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<b>(21) International Application Number:</b> PCT/US92/02892 <b>(22) International Filing Date:</b> 9 April 1992 (09.04.92)  <b>(30) Priority data:</b> 832,211 6 February 1992 (06.02.92) US  <b>(71) Applicant:</b> DANA-FARBER CANCER INSTITUTE, INC. [US/US]; 44 Binney Street, Boston, MA 02115 (US).  <b>(72) Inventors:</b> MORIMOTO, Chikao ; 329 Great Plain Avenue, Needham, MA 02192 (US). SCHLOSSMAN, Stuart, F. ; One Fox Place, Newton, MA 02159 (US). TANAKA, Toshiaki ; 17 Shepard Park, Newton, MA 02168 (US).		<b>(74) Agent:</b> FRASER, Janis, K. ; Fish & Richardson, 225 Franklin Street, Boston, MA 02110-2804 (US).  <b>(81) Designated States:</b> CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> HUMAN CD26 AND METHODS FOR USE  <b>(57) Abstract</b>  A polypeptide fragment of CD26 (or analogs thereof) capable of disrupting the naturally-occurring binding interaction between CD45 and CD26, and a method of screening such compounds to identify compounds capable of inhibiting the binding of CD26 to CD45, which method includes the steps of: a) providing a first and a second sample of cells expressing both CD26 and CD45; b) incubating the first sample in the presence of a candidate compound; c) incubating the second sample in the absence of the candidate compound; d) generating a first immunoprecipitate by adding to the first sample a first aliquot of an anti-CD26 antibody; e) generating a second immunoprecipitate by adding to the second sample a second aliquot of the antibody; and f) determining whether the amount of CD45 present in the first immunoprecipitate is less than the amount of CD45 present in the second immunoprecipitate, the presence of a lesser amount of CD45 in the first immunoprecipitate than in the second immunoprecipitate indicating that the candidate compound inhibits the binding.		

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HUMAN CD26 AND METHODS FOR USEBackground of the Invention

The field of the invention is human T cell activation antigens.

CD26 is a human T cell activation antigen originally identified by its reactivity with the  
5 monoclonal antibody Ta1 (Fox et al., *J. Immunol.* 133:1250, 1984). CD26 has recently been shown to be identical to human dipeptidyl peptidase IV (EC 3.4.14.5) (Ulmer et al., *Scand. J. Immunol.* 31:429, 1990; Barton et al., *J. Leukocyte Biol.* 48:291, 1990). Dipeptidyl  
10 peptidase IV (DPPIV) is a serine exopeptidase which is capable of cleaving x-proline or x-alanine (where x is any amino acid) from the amino terminus of certain peptides.

CD26 is recognized by a second monoclonal  
15 antibody, anti-1F7 (Morimoto et al., *J. Immunol.* 143:3430, 1989). Dang et al. (*J. Immunol.* 144:4092, 1990) report that solid phase-immobilized anti-1F7 mAb is capable of inducing proliferation of human CD4<sup>+</sup> T lymphocytes in conjunction with submitogenic doses of  
20 anti-CD3 or anti-CD2 antibodies. They suggest that the CD26 antigen is involved in CD3- and CD2-induced human CD4<sup>+</sup> T cell activation.

Summary of the Invention

In one aspect, the invention features a  
25 polypeptide fragment of CD26 having an amino acid sequence substantially identical to the amino acid sequence of CD26, except that amino acid residues 3-9 of the latter sequence have been deleted ( $\Delta$ 3-9, SEQ ID NO: 2). In preferred embodiments, the polypeptide has an

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amino acid sequence identical to the amino acid sequence of SEQ ID NO: 2; the polypeptide is soluble under physiological conditions; and the polypeptide is substantially pure. Also within the invention is the  
5 product of signal peptidase proteolytic cleavage of this polypeptide, which would be a form of CD26 lacking residues 1-34, 1-35, 1-36, or 1-37.

In a related aspect, the invention features a nucleic acid encoding a polypeptide fragment of CD26  
10 having an amino acid sequence substantially identical to the amino acid sequence of  $\Delta 3-9$  (SEQ ID NO: 2). In another related aspect, the invention features a plasmid which includes this nucleic acid, and preferably also an expression control sequence.

15 In another aspect, the invention features a polypeptide fragment of CD26 having an amino acid sequence substantially identical to the amino acid sequence of CD26 except that residues 24-34 of the latter sequence are deleted ( $\Delta 24-34$ , SEQ ID NO: 3). In  
20 preferred embodiments, the polypeptide has an amino acid sequence identical to the amino acid sequence of SEQ ID NO: 3; the polypeptide is soluble under physiological conditions; and the polypeptide is substantially pure. Also within the invention is the product of signal  
25 peptidase proteolytic cleavage of this polypeptide, which would be a form of CD26 lacking residues 1-34, 1-35, 1-36, or 1-37.

In a related aspect, the invention features a nucleic acid encoding a polypeptide fragment of CD26  
30 having an amino acid sequence substantially identical to the amino acid sequence of SEQ ID NO: 3 ( $\Delta 24-34$ ). In another related aspect, the invention features a plasmid which includes the nucleic acid, and preferably also an expression control sequence.

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Polypeptide fragments of CD26 which are soluble under physiological conditions generally lack most or all of the hydrophobic amino acid residues found near the amino terminus of the polypeptide depicted in SEQ ID NO:

- 5 1. This can be accomplished by genetically manipulating a nucleic acid encoding CD26 to delete the hydrophobic residues, or to delete enough of the N-terminal amino acids (e.g., residues 3-9 or 24-34) to leave the resulting polypeptide susceptible to cleavage by signal  
10 peptidase. Other fragments of CD26 which are within the invention include those in which all or part of the putative dipeptidyl aminopeptidase catalytic site (Gly<sub>627</sub> to Gly<sub>631</sub>) is deleted. Such fragments, which include *inter alia* the deletion mutant shown in Fig. 15 (SEQ ID  
15 NO: 11); fragments having additional deletions such as those in  $\Delta$ 3-9 (SEQ ID NO: 2) and  $\Delta$ 24-34 (SEQ ID NO: 3); and those missing the entire signal peptide region up to Ala<sub>35</sub>, Thr<sub>36</sub>, Ala<sub>37</sub> or Asp<sub>38</sub>, would constitute enzymatically inactive fragments of CD26 useful in the  
20 screening assays of the invention, as well as for inhibiting complex formation between CD26 and/or CD45 and p43.

- By "substantially pure" is meant a polypeptide or protein which has been separated from biological  
25 macromolecules, (e.g., other proteins, carbohydrates, etc.) with which it naturally occurs. Typically, a protein or polypeptide of interest is substantially pure when less than 25% (preferably less than 15%) of the dry weight of the sample consists of such other  
30 macromolecules.

By "physiological conditions" is meant an aqueous solution, whether *in vivo* or *in vitro*, having a pH and salt concentration similar to that found in serum. Phosphate buffered saline is an example of a commonly

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used buffer in which a polypeptide that is soluble under physiological conditions would be soluble.

By "substantially identical to CD26" is meant that at least 80%, preferably at least 90%, more preferably at least 95%, most preferably at least 99%, of the amino acid sequence is identical to that of the corresponding portion of CD26, and any non-identical amino acids in the sequence are amino acid substitutions, preferably conservative, which do not eliminate the biological activity of the molecule.

By "plasmid" is meant an extrachromosomal DNA molecule which includes sequences that permit replication within a particular host cell.

By "expression control sequence" is meant a nucleotide sequence which includes recognition sequences for factors that control expression of a protein coding sequence to which it is operably linked. Accordingly, an expression control sequence generally includes sequences for controlling both transcription and translation: for example, promoters, ribosome binding sites, repressor binding sites, and activator binding sites.

In another aspect, the invention features a polypeptide fragment of CD26 (or analogs thereof) capable of disrupting the naturally-occurring binding interaction between CD45 and CD26. The term "analogs" refers to polypeptide fragments of CD26 having conservative and/or non-conservative substitutions for some of the amino acids of naturally-occurring CD26, having D-amino acids in place of some or all of the corresponding L-amino acids, or having non-peptide bonds in place of some of the peptide bonds of CD26. Techniques for producing such analogs are well known in the art, and can be readily accomplished by those of ordinary skill. Preferably at least 85%, more preferably at least 95%, and most preferably at least 99%, of the amino acids in the analog

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are identical to the corresponding ones in CD26. It is important that the substitutions do not eliminate the ability of the polypeptide fragment to interfere with the naturally occurring association between CD26 and CD45.

5 In some instances, the removal of peptide bonds from a polypeptide compound is a desirable goal because the presence of such bonds may leave the compound susceptible to attack by proteolytic enzymes. Additionally, such peptide bonds may affect the biological availability of  
10 the resulting therapeutic molecules. The removal of peptide bonds is part of a process referred to as "depeptidization". Depeptidization entails such modifications as replacement of the peptide bond (-CONH-) between two given amino acids with a spatially similar  
15 group such as -CH<sub>2</sub>CH<sub>2</sub>-, -CH<sub>2</sub>-O-, -CH=CH- or -CH<sub>2</sub>S-, generally by incorporating a non-peptide mimetic of the dipeptide into the chemically synthesized analog of the invention.

Polypeptides and analogs which disrupt the  
20 interaction between CD26 and CD45 can be identified using the immunoprecipitation assay described herein below.

In another aspect, the invention features a method for screening candidate compounds to identify compounds capable of inhibiting the binding of CD26 to CD45, which  
25 method includes the steps of:

(a) providing a first and a second sample of cells expressing both CD26 and CD45;

(b) incubating the first sample in the presence of a candidate compound;

30 (c) incubating the second sample in the absence of the candidate compound;

(d) generating a first immunoprecipitate by adding to the first sample a first aliquot of an anti-CD26 antibody;

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(e) generating a second immunoprecipitate by adding to the second sample a second aliquot of the antibody; and

(f) determining whether the amount of CD45 present in the first immunoprecipitate is less than the amount of CD45 present in the second immunoprecipitate, the presence of a lesser amount of CD45 in the first immunoprecipitate than in the second immunoprecipitate indicating that the candidate compound inhibits the binding.

As used herein, an anti-CD26 antibody is one capable of forming a specific immune complex with CD26, i.e., the antibody binds directly to CD26 but does not substantially bind directly to other molecules in the assay of the invention.

In another aspect, the invention features a method for screening candidate compounds to identify compounds capable of inhibiting the binding of CD26 to CD45, which method includes the steps of:

(a) providing a first and a second sample of cells expressing both CD26 and CD45;

(b) incubating the first sample in the presence of a candidate compound;

(c) incubating the second sample in the absence of the candidate compound;

(d) generating a first immunoprecipitate by adding to the first sample a first aliquot of an anti-CD45 antibody;

(e) generating a second immunoprecipitate by adding to the second sample a second aliquot of the antibody; and

(f) determining whether the amount of CD26 present in the first immunoprecipitate is less than the amount of CD26 present in the second immunoprecipitate, the presence of a lesser amount of CD26 in the first

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immunoprecipitate than in the second immunoprecipitate indicating that the candidate compound inhibits the binding.

In another aspect, the invention features a  
5 monoclonal antibody which, when contacted under physiological conditions with a cell (preferably a eukaryotic cell such as a mammalian cell) expressing CD26 and CD45, interferes with the association of CD26 and CD45; and a method for assaying for such an antibody.

10 In yet another aspect, the invention features a method which includes:

(a) providing a cell which expresses CD45 on its surface; and

(b) introducing into the cell a nucleic acid  
15 encoding CD26, such that the cell expresses CD26 on its surface.

In yet another aspect, the invention features a method which includes:

(a) providing a cell which expresses CD26 on its  
20 surface; and

(b) introducing into the cell a nucleic acid encoding CD45, such that the cell expresses CD45 on its surface.

In other aspects, the invention includes a cell  
25 transfected with a nucleic acid encoding CD26, the cell expressing both CD26 and CD45 on its surface; and a cell transfected with a nucleic acid encoding CD45, the cell expressing both CD26 and CD45 on its surface. In preferred embodiments, the cells are T-cells such as  
30 Jurkat cells.

In another aspect, the invention features a method which includes:

(a) providing a cell which expresses neither CD26 nor CD45 on its surface; and

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(b) transfecting the cell with a nucleic acid encoding CD26 and a nucleic acid encoding CD45.

In yet another aspect, the invention includes a method of generating a hybridoma cell, which method  
5 includes:

(a) providing a cell transfected with nucleic acid encoding CD26, such that the cell expresses CD26 on its surface;

(b) using the cell as an antigen to induce an  
10 immune response in a subject animal; and

(c) fusing a B lymphocyte from the subject animal with a cell from an immortal cell line (i.e., a line of cells which can be maintained indefinitely in culture) to produce a hybridoma cell.

15 In a related aspect, the invention features a hybridoma cell generated by:

(a) providing a cell transfected with nucleic acid encoding CD26, such that the cell expresses CD26 on its surface;

20 (b) using the cell as an antigen to induce an immune response in a subject animal; and

(c) fusing a B lymphocyte from the subject animal with a cell from an immortal cell line to produce a hybridoma cell, wherein the hybridoma cell produces a  
25 monoclonal antibody specific for CD26. Applicable methods of inducing an immune response in an animal by using cells as the antigen, and fusing B lymphocytes with immortal cells to produce hybridoma cells, are well known to those of ordinary skill in the art of making  
30 hybridomas. The resulting hybridomas are then cloned and screened for production of monoclonal antibodies which bind to cells expressing the CD26 antigen, but not to identical cells which do not express the CD26 antigen.

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Also within the invention are cell-free preparations of CD26, or a fragment thereof, complexed with CD45, or a fragment thereof. Such complexes may be conveniently prepared by recombinant expression of each of the relevant polypeptides in a manner that prevents their being anchored to the cellular membrane (e.g., by use of a soluble fragment of each), or by isolation of the full-length proteins from a cell membrane preparation, and by combining the two polypeptides to form the desired complex either before or after removal of contaminating cellular constituents. Such complexes would be useful, e.g., for generating monoclonal antibodies specific for the complex, and for screening for compounds capable of interfering with the association of CD26 and CD45.

Also within the invention are purified preparations of p43, a 43 kDa molecule which, like CD45, associates with CD26 in cells and therefore is thought to play a role in T cell activation, and cell-free preparations of CD26 (or a fragment thereof) complexed with p43 (or a fragment thereof). The screening assay described above for compounds capable of inhibiting the interaction of CD26 and CD45 can be readily adapted to detect compounds (including fragments of CD26 or p43) capable of inhibiting the interaction of CD26 and p43.

CD26 is known to play a role in T cell activation. By interfering with the normal functioning of CD26, one can control the process of T cell activation, and thus prevent such unwanted immune responses as transplant rejection and certain autoimmune diseases. The information disclosed herein concerning proteins with which CD26 associates on the T cell provides the means for designing and screening compounds that interfere with CD26 function in the cell.

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Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

#### Detailed Description

5 The drawings are first briefly described.

#### Drawings

Fig. 1 depicts the nucleotide sequence and deduced amino acid sequence (SEQ ID NO:1) of the cDNA clone for human CD26.

10 Fig. 2 depicts the results of an indirect fluorescence staining assay.

Fig. 3 is a pair of photographs of gels illustrating the results of immunoprecipitation analysis (panel A) and enzymatic activity analysis (panel B).

15 Fig. 4 is a set of graphs depicting the results of a  $[Ca^{2+}]_i$  mobilization assay.

Fig. 5 is a graph illustrating the effect of various treatments on interleukin-2 production.

20 Fig. 6 is a photograph of a gel illustrating the results of immunoblotting analysis.

Fig. 7 depicts the results of FACS analysis.

Figs. 8-12 are photographs of gels illustrating the results of immunoprecipitation assays.

25 Fig. 13 is a representation of the amino acid sequence of CD26 in which the deleted amino acids of  $\Delta 3-9$  (SEQ ID NO: 2) are indicated by a box, and the probable proteolytic cleavage sites of the signal peptidase are indicated by arrows.

30 Fig. 14 is a representation of the amino acid sequence of CD26 in which the deleted amino acids of  $\Delta 24-34$  (SEQ ID NO: 3) are indicated by a box, and the probable proteolytic cleavage sites of the signal peptidase are indicated by arrows.

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Fig. 15 depicts the amino acid sequence of a CD26 fragment lacking a portion of the carboxy terminal region of CD26 (SEQ ID NO: 11).

#### Sequencing and Characterization of CD26

5 Described below is the cloning and sequencing of a full-length CD26 cDNA. Also described are a series of experiments which demonstrate that: (1) modulation of CD26 from the surface of T lymphocytes leads to enhanced CD3 $\xi$  phosphorylation and increased CD4-associated p56<sup>lck</sup>  
10 tyrosine kinase activity; (2) CD26 is comodulated with CD45; and (3) CD26 and CD45 are closely associated.

#### Cells and Antibodies

Human peripheral blood mononuclear cells (PBMC), E rosette-positive cells and PHA-activated T cells for  
15 use in the experiments described below were prepared as follows. Human PBMC were isolated from healthy volunteer donors by Ficoll-Hypaque density gradient centrifugation (LKB Biotechnology, Inc., Piscataway, NJ). Unfractionated mononuclear cells were separated into E  
20 rosette-positive (E+) and E rosette-negative (E-) populations, and the E+ cells were depleted of contaminating monocytes as described (Morimoto et al., *J. Immunol.* 134:3762, 1985; Morimoto et al., *J. Immunol.* 134:1508, 1985; Matsuyama et al., *J. Exp. Med.* 170:1133,  
25 1989). These T cells were used for experiments involving T cells in this report. E+ cells were stimulated with PHA (0.25  $\mu$ g/ml) and rIL-2 (40 U/ml) for 7 days in RPMI 1640 medium supplemented with 10% human AB serum, 4mM L-glutamine, 25 mM HEPES buffer, 0.5% sodium bicarbonate,  
30 and 1% penicillin/streptomycin (culture medium) and used as PHA blasts. The monoclonal antibodies used were anti-CD26 (Ta1/4EL-1C7, IgG<sub>1</sub>; 1F7, IgG<sub>1</sub>; 5F8, IgG<sub>1</sub>), and anti-CD3 (T3/RW24B6; IgG<sub>2b</sub>) (Fox et al., *J. Immunol.* 133:1250, 1984; Morimoto et al., *J. Immunol.* 143:3430, 1989;

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Morimoto et al., *J. Immunol.* 134:3762, 1985). Anti-CD29 (4B4; IgG<sub>1</sub>) (Morimoto et al., *J. Immunol.* 134:3762, 1985) was used as an isotype-matched control throughout.

Isolation of a CD26 cDNA

5 To isolate a CD26 cDNA, a cDNA library was constructed from mRNA isolated from human PHA-activated T cells using the CDM7 vector. Briefly, poly(A)+ RNA was prepared from 4-day-old PHA-activated T cells by the guanidinium isothiocyanate method (Chirgwin et al.,  
10 *Biochem.* 18:5294, 1979), and an expression library was prepared as previously described, except that the vector CDM7, a precursor to CDM8 lacking polyoma sequences, was employed (Aruffo et al., *Proc. Natl. Acad. Sci. USA*  
84:8573, 1987; Seral et al., *Proc. Natl. Acad. Sci. USA*  
15 87:3365, 1987). Recombinant hybrid plasmids were transfected into COS cells, and CD26 expressing cells were immunoselected with the monoclonal antibody, anti-Tal (Aruffo et al., *supra*; Seed et al., *supra*). Reactive cells were retained on antibody coated dishes, and  
20 plasmids were recovered from transfected cells. Plasmid DNAs were further selected by three additional rounds of transfection and immunoselection. Two of eight clones thus isolated were found to encode anti-Tal reactive determinants. The two clones were identical by  
25 restriction enzyme fragment mapping.

Sequencing of both strands of the isolated 2.9 kb CD26 cDNA by the dideoxy sequencing method revealed a 2298 base pair open reading frame beginning with an ATG at nucleotide 11 which conforms to consensus  
30 translational initiation sites (Fig. 1). The deduced CD26 structure is a 766 amino acid residue polypeptide with a molecular weight of approximately 88,300 (SEQ ID NO: 1).

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Predicted Structure of CD26

The predicted CD26 polypeptide has a single stretch of hydrophobic amino acids in the N-terminal region between residues 7 and 28 (Fig. 1, boxed), which is sufficiently long and hydrophobic to span a lipid bilayer (Davis et al., *Cell* 41:607, 1985). The sequence is preceded by six N-terminal residues which contain polar and charged residues, and is followed by charged residues that would not allow cleavage by signal peptidase (von Heijne, *Nucl. Acids Res.* 14:4683, 1986). This sequence thus has the characteristics of a signal sequence of a type II membrane protein, which serves both to direct the translocation of the nascent protein across the membrane of the rough endoplasmic reticulum, and to anchor the mature protein in the membrane (Hong et al., *supra*, 1990; Shipp et al., *Proc. Natl. Acad. Sci. USA* 85:4819, 1988; Thomas et al., *J. Clin. Invest.* 83:1299, 1989). Furthermore, the fact that potential N-glycosylation sites are located in the carboxy side of the hydrophobic core (Fig. 1, short underlines) suggests that CD26 is a type II membrane protein. Therefore, the N-terminal 6 amino acid residues are predicted to be cytoplasmic, and the next 22 amino acids, which are primarily hydrophobic, are predicted to transverse the cytoplasmic membrane. The 738 C-terminal amino acids constitute the predicted extracellular domain of CD26.

The predicted extracellular domain of CD26 may be conveniently divided into three regions: an N-terminal glycosylated region (residues 29 to 323), a relatively cysteine-rich middle section (residues 324 to 551), and a C-terminal region (residues 552 to 766) (Fig. 1). The N-terminal region contains 8 of the 10 potential attachment sites for N-linked glycans (Fig. 1, short underlines) (Marshall, *Ann. Rev. Biochem.* 41:673, 1972), and one of the 12 cysteine residues (Fig. 1, asterisks). In

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contrast, the subsequent cysteine-rich section contains 9  
cysteines but only one N-linked glycosylation site. The  
C-terminal region contains two cysteines, one N-linked  
glycosylation site and a potential catalytic site (Fig.  
5 1, double underline), the sequence G-W-S-Y-G at position  
627 to 631. This sequence fits the consensus G-X-S-X-G  
found in the active sites of serine proteases and  
esterases, although tryptophan and tyrosine flanking the  
catalytic serine are unusual residues at these positions  
10 (Brenner, *Nature* 334:528, 1988).

Homology with the Other Proteins.

The predicted amino acid sequence of the human  
CD26 antigen (SEQ ID NO: 1) is 85% homologous to the  
deduced rat DPPIV enzyme sequence predicted from cDNAs  
15 isolated from rat liver and kidney libraries.  
Considering this high degree of homology and the fact  
that anti-Tal antibody reacts with human liver and kidney  
epithelium (Mobius et al., *Exp. Immunol.* 74:431, 1988),  
the DPPIV enzyme present in those tissues is probably the  
20 functional counterpart of the CD26 antigen. This high  
degree of homology also supports the prediction of the  
membrane topology of CD26, because rat DPPIV has been  
shown to be a type II membrane protein (Hong et al.,  
*supra* 1990).

25 Aside from the signal sequence, the greatest  
homology between rat (Ogata et al., *supra*) and human  
CD26/DPPIV proteins is in the C-terminal region, which  
includes the putative catalytic site. In fact, the  
sequences are identical from residues 624 to 724, and 94%  
30 homologous from residues 552 to 766. This C-terminal  
region is 46% homologous to a region of the predicted  
yeast aminopeptidase B (DPAPB) sequence (Roberts et al.,  
*J. Cell. Biol.* 108:1363, 1989). Further, CD26 amino acid  
residues 107 to 233 are 36% homologous to DPAPB. The  
35 yeast DPAPB enzyme is also a type II membrane dipeptidyl

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aminopeptidase, and is involved in the maturation of the yeast pheromone alpha factor. The putative catalytic sequence G-W-S-Y-G is conserved between human and rat CD26/DPPIV and yeast DPAPB.

- 5           Recently the structures for CD10 and CD13 were determined by cDNA cloning (Shipp et al., *supra*, Thomas et al., *supra*). These antigens are ectoenzymes which have neutral endopeptidase [EC. 3.4.24.11] and aminopeptidase N [EC. 3.4.11.2] activities, respectively.
- 10   Although CD10 and CD13 are also type II membrane proteins, there is no significant sequence homology between these enzymes and CD26.

- Although the CD26 antigen is known to be a functional collagen receptor (Dang et al., *J. Exp. Med.* 172:649, 1990), a homology search did not find significant homology with any other known collagen-binding proteins such as fibronectin, CD11b and the integrins.

15           Characterization of CD26 Antigen expressed on Transfected Jurkat Cells

- 20           To characterize the cDNA-encoded CD26 antigen, the human T cell leukemia line, Jurkat, was transfected with the expression plasmid pSR $\alpha$ 26, in which the CD26 cDNA was placed under the control of the SR $\alpha$  promoter. Briefly, the CD26 cDNA insert was cloned into the PstI and EcoRI sites of the plasmid pCDLSR $\alpha$ 296 (Takebe et al., *Mol. Cell. Biol.* 8:466, 1988) by blunt-end ligation to create the CD26 expression plasmid, pSR $\alpha$ -26. pSR $\alpha$ -26, digested with SalI, and pSV2neo-SP (confers neomycin resistance to host cells; Streuli et al., *EMBO J.* 8:787, 1989), digested with PvuI, were used to co-transfect Jurkat cells according to Streuli et al. (*supra*). Transfectants were initially selected in RPMI1640 supplemented with 10% fetal calf serum, 4mM glutamine and 1.0 mg/ml Geneticin (Gibco/BRL, Bethesda, MD). Subsequently, the

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concentration of Geneticin was gradually decreased to 0.25 mg/ml during the selection period. Geneticin-resistant clones were further screened for CD3 and CD26 antigen expression by cell-surface staining as described 5 below. Transfectants were maintained in the above medium containing 0.25 mg/ml Geneticin.

Staining of cell surface antigens with monoclonal antibodies and flow cytometry analyses using an EPICS V cell sorter (Coulter) were performed as described by Dang 10 et al. (*J. Immunol.* 144:4092, 1990).

Parental Jurkat cells do not express detectable amounts of the CD26 antigen as determined by cell surface staining (Fig. 2), or by a binding assay with radiolabeled Tal monoclonal antibody. Northern blotting 15 analysis revealed that this cell line also does not express CD26 mRNA even after phorbol 12-myristate 13-acetate (PMA) treatment, which is known to induce CD26 expression (Dang et al., *J. Immunol.* 145:3963, 1990). Referring to Fig. 2, the Jurkat-CD26 transfectant 26.C28 20 had high expression of the CD26 antigen. On the other hand, another Jurkat-CD26 clone, 26.24, expressed only moderate levels of the antigen. Both transfectants were reactive with three anti-CD26 monoclonal antibodies (Tal, 1F7, and 5F8) which define three distinct CD26 antigen 25 epitopes.

To study whether the CD26 antigen expressed on Jurkat T cell lines had the same characteristics as that on peripheral blood lymphocytes, immunoprecipitation experiments were carried out.

30 Briefly, cell surface proteins were labelled with lactoperoxidase-catalyzed iodination as described by Morimoto et al., (*J. Immunol.* 143:3430, 1989). Immunoprecipitations (employing an NP-40 lysis buffer) using 1F7 monoclonal antibody were performed as described 35 by Morimoto et al. (*supra*, 1989). Immunoprecipitated

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proteins were separated by 8% SDS-PAGE under reducing conditions.

Referring to Fig. 3 (panel A), 1F7 monoclonal antibody immunoprecipitated a 110 kDa protein from the CD26 transfected Jurkat cells (lanes 2 and 3) as well as from PHA blasts (lane 4). There was no detectable 110 kDa band in nontransfected (lane 1) and vector-only transfected Jurkat cells. Control anti-4B4 monoclonal antibody immunoprecipitated a comparable amount of 130 kDa protein from each of the cell lines. Interestingly, 1F7 immunoprecipitated an additional 43 kDa protein from both transfectants and PHA blasts. Similar results were observed using peripheral blood T cells. This 43 kDa protein may contribute to T cell activation through its association with CD26.

DPP-IV enzymatic activity was measured using an Enzyme Overlay Membrane system (EOM, Enzyme System Products, Dublin, CA). Briefly, lysates were incubated with SDS sample buffer for 1 hr at room temperature and separated by SDS-PAGE under non-reducing conditions. Following electrophoresis, the EOM moistened with 0.5M Tris-HCl, pH 7.8, was placed on the surface of the gel and this sandwich was incubated for 20 min in a humidified box at 37°C. The reaction was monitored by long wavelength ultraviolet light. Referring to Fig. 3, panel B, DPPIV enzymatic activity was associated with a 160 kDa protein in both transfectants (lanes 2 and 3) and PHA blasts (lane 4), but not in parental Jurkat cells (lane 1), or vector-only transfected cells. It should be noted that the DPPIV enzyme activity was stable in both non-reducing and reducing conditions but disappeared after boiling of the samples. While the apparent molecular weight of CD26 was 160,000 for preparations that were not boiled prior to electrophoresis, the molecular weight of CD26 antigen was 110,000 if the

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protein was boiled prior to SDS-PAGE analysis. Similar results have been reported for rat hepatocyte DPPIV (Walburg et al., *Exp. Cell. Res.* 158:509, 1985). Taken together, the above-described results indicate that the

5 CD26 antigen expressed on the transfected Jurkat cells was the same as that on peripheral blood T cells.

Functional Analysis of CD26 Antigen on Jurkat Transfectants

To determine whether the CD26 antigen expressed on

10 transfected Jurkat cells has biological activity similar to that of CD26 expressed on peripheral blood T cells, we examined  $[Ca^{2+}]_i$  mobilization induced by CD26 antigen triggering.

Briefly, loading of indo-1 pentaacetoxymethyl

15 ester (Calbiochem, San Diego, CA) into cells and the measurement of its fluorescence by flow cytometry were performed as described by (Blue et al., *J. Immunol.* 140:376, 1988). Indo-1-loaded cells were preincubated for 1-2 minutes with antibodies and the basal

20 intracellular calcium levels were determined for 33 seconds before the addition of polyclonal goat anti-mouse antibody (10  $\mu$ g/ml) (Tago, Burlingame, CA). The RW24B6 anti-CD3 antibody was titrated in this system to determine the submitogenic dose for triggering each cell

25 type. After preincubation of each transfectant with anti-CD26 and/or a submitogenic dose of anti-CD3, anti-mouse antibody was added (time point of addition indicated by small arrows in Fig. 4). Antibody concentrations were 1  $\mu$ g/ml for anti-1F7 and 20 ng/ml for

30 anti-CD3.

Referring to Fig. 4, crosslinking of anti-CD26 and submitogenic doses of anti-CD3 with goat anti-mouse immunoglobulin on CD26 transfectants resulted in greater  $[Ca^{2+}]_i$  mobilization than crosslinking of anti-CD3 alone.

35 These antibodies did not induce  $[Ca^{2+}]_i$  mobilization

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without cross-linking. It is well known that the  $[Ca^{2+}]_i$  mobilization signal is divided into two phases: the initial transient rise, and the sustained increase phase (Gardner, *Cell* 59:15, 1989; Goldsmith et al., *Science* 240:1029, 1988). For both CD26 transfectants, the anti-CD26 and anti-CD3 crosslinking induced a strong initial  $[Ca^{2+}]_i$  increase (Fig. 4). In addition, for the clone 26.C28, crosslinking induced a sustained increase of the  $[Ca^{2+}]_i$  level as well (Fig. 4). The differential pattern of  $[Ca^{2+}]_i$  mobilization of the two transfectants may be attributed to the difference in the amount of CD26 antigen expressed by these two transfectants. The enhanced  $[Ca^{2+}]_i$  mobilization was specific because, as was reported for peripheral blood T cells (Dang et al., *J. Immunol.* 145:3963, 1990), crosslinking of the CD26 antigen alone did not induce  $[Ca^{2+}]_i$  mobilization. Furthermore, crosslinking of anti-CD26 and anti-CD3 did not enhance the  $[Ca^{2+}]_i$  mobilization of nontransfected or vector-only transfected Jurkat cells, and crosslinking of the isotype-matched control antibody, anti-4B4, did not result in enhanced  $[Ca^{2+}]_i$  mobilization of the transfectants. Similar to the data observed with transfectants, a small but significant transient rise in  $[Ca^{2+}]_i$  mobilization was observed in normal resting T cells following CD26 and CD3 crosslinking.

IL-2 production by transfected cells cultured in antibody-coated plates was measured as described by Dang et al., *J. Immunol.* 144:4092, 1990), except that the cell concentration was adjusted to  $2 \times 10^6$  cell/ml. After 24 hr of culture, supernatants were assayed for IL-2 production using ELISA (R&D system, Minneapolis, MN). Referring to Fig. 5, incubation of the clone 26.C28 transfectants with solid-phase-immobilized anti-1F7 and anti-CD3, which mimicked the crosslinking by anti-mouse antibody, induced the production of a significant amount of IL-2 (striped

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bar), as compared to the control, vector-only transfected, Jurkat cells (solid bar). These results indicate that the CD26 Jurkat transfectants were functionally similar to peripheral blood T cells.

- 5 Moreover, the above data indicate that the stimulatory effect of anti-CD26 and anti-CD3 crosslinking in T cells was in part mediated by an enhancement of  $[Ca^{2+}]_i$  mobilization. Since it is well known that the transient rise, as well as the sustained increase, in  $[Ca^{2+}]_i$  is  
10 necessary for IL-2 production (Gardner, *supra*; Goldsmith, *supra*), the sustained increase of the  $[Ca^{2+}]_i$  observed in clone 26.C28 may be the basis for enhanced IL-2 production seen with the transfectant following anti-CD26 and anti-CD3 stimulation. Thus, the data obtained using  
15 Jurkat CD26 transfectants provide direct evidence that the CD26 antigen plays an integral role in T cell activation.

#### Co-association of CD26 and CD45

- The experiments described below demonstrate that  
20 modulation of CD26 on the surface of T lymphocytes by anti-CD26 monoclonal antibody leads to enhanced phosphorylation of CD3 and increased  $p56^{lck}$  tyrosine kinase activity. Modulation experiments described below demonstrate that CD26 is co-modulated with CD45.

- 25 Finally, immunoprecipitation assays described below demonstrate that CD26 and CD45 are closely associated. Taken together, the results indicate that an interaction between CD26 and CD45 increases  $p56^{lck}$  tyrosine kinase activity, CD3 chain phosphorylation, and T lymphocyte  
30 activation.

#### Enhancement of CD3 $\zeta$ Phosphorylation Following anti-CD26 (1F7) Treatment

To evaluate the effect of anti-CD26 antibodies on one of the earliest signaling events in T cell

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activation, we investigated their role in the tyrosine phosphorylation of CD3 $\zeta$ .

Immunoblotting analysis of tyrosine phosphorylation of CD3 $\zeta$  was performed as described by Vivier et al. (*J. Immunol.* 146:206, 1990). Briefly, peripheral blood T cells ( $10 \times 10^6$  per sample) were incubated in culture media alone or with anti-CD26 (1F7; 1:100 ascites dilution) for various times at 37°C. Cells were then extensively washed in ice cold PBS containing 5mM EDTA, 10mM NaF, 10mM sodium pyrophosphate, and 0.4mM sodium vanadate, then solubilized in lysis buffer (1% NP-40, 150mM NaCl, 50mM Tris HCl, pH 8.0, 5mM EDTA, 1mM PMSF, 10mM iodoacetamide, 10mM NaF, 10mM sodium pyrophosphate, 0.4mM sodium vanadate) for 15 min on ice. After removing insoluble material by centrifugation at 12,000 rpm for 15 min, samples were combined with an equal volume of sample buffer (2% SDS, 10% glycerol, 0.1M Tris [pH 6.8] 0.02% bromophenol blue), reduced with 5% 2-mercaptoethanol, and separated on 12% SDS-polyacrylamide gels. After separation on SDS-PAGE, cell lysates were transferred to nitrocellulose, and developed using  $^{125}\text{I}$ -labelled anti-phosphotyrosine (UBI, NY; 100,000 cpm/ml in PBS containing 1% BSA). Affinity-purified anti-phosphotyrosine was iodinated to a specific radioactivity of 10-20  $\mu\text{Ci}/\mu\text{g}$  protein using iodobeads (Pierce Chemical Co., Rockford, IL).

Referring to Fig. 6, a 21 kD tyrosine phosphoprotein (p21), which has been previously identified in T cells stimulated with various stimuli as phosphorylated CD3 $\zeta$  (Vivier et al., *supra*, 1990; Vivier et al., *J. Immunol.* 146:1142, 1991; Ashwell et al., *Annu. Rev. Immunol.* 8:139, 1990), was detected at a constitutive level in samples not treated with anti-CD26 (lane 1). Anti-CD26 treatment significantly increased the phosphorylation of CD3 $\zeta$  over the constitutive level

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after 1 hour of anti-CD26 incubation (lane 2). The level of phosphorylated CD3 $\zeta$  gradually increased with time, reaching a maximum level after 4 hours of anti-CD26 incubation (lanes 3 and 4; 2 and 4 hours of anti-CD26 treatment respectively), and gradually decreased upon longer incubation (lanes 5 and 6; 6 and 8 hours of anti-CD26 treatment respectively). The total amount of CD3 $\zeta$  chain (phosphorylated and non-phosphorylated) present, determined by immunoblotting the same membrane with an anti-CD3 $\zeta$  mAb, was similar in all samples. Although anti-CD26 by itself can not induce T cell proliferation, these results show that CD26 modulation provides an initial T cell activation signal as measured by enhanced CD3 $\zeta$  phosphorylation.

15 Comodulation of CD26 and CD45 by anti-CD26 Antibody (1F7)  
Treatment

The fact that the cytoplasmic domain of CD26 (DPPIV) in the rat includes only six amino acid residues suggests that CD26 might be associated with another molecule which acts in a signal transducing capacity, as has been found in the case of the IL-6 receptor and the IL-2 (p55) receptor (Taga et al., *Cell* 58:573, 1989; Robb et al., *J. Exp. Med.* 165:1201; 1987). The experiments described below indicate that CD26 is associated with another cell surface molecule, CD45. Human peripheral blood T cells were used in the experiments described below and obtained as described by Dang et al. *J. Immunol.* 144:4092, 1990. Anti-CD26 (1F7) induced modulation was performed as previously described (by Dang et al. *J. Immunol.* 145:3963, 1990). Briefly, peripheral blood T cells were incubated overnight at 37°C in medium containing anti-CD26 (1F7) at 1:100 ascites dilution. Cells were then collected, washed and stained with anti-CD26 (1F7) and FITC-conjugated goat anti-mouse IgG; or they were stained with anti-CD45RA (2H4)-PE, anti-CD2-PE,

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anti-CD3-PE (Coulter) or biotinylated anti-CD45RO (UCHL-1) and PE-conjugated avidin.

Flow cytometry analysis was performed using an Epics V cell sorter (Coulter Electronics) as previously described (Morimoto et al., *J. Immunol.* 143:3430, 1989).

The negative control of each fluorescence was less than 5%. The FACS analysis presented in Fig. 7 are representative of three separate experiments. As shown in Fig. 7, overnight incubation with anti-CD26 led to a significant reduction in CD26 expression on T cells. Interestingly, while CD26 modulation did not have any detectable effect on CD2, CD3 or CD45RA expression, the expression of CD45RO, particularly the high fluorescence peak of CD45RO, was markedly reduced. In addition, modulation of CD2, CD3, or CD4 with respective antibodies had no effect on CD45RO expression. Thus, the co-modulation of CD45RO induced by anti-CD26 treatment appears to be specific for this structure.

Co-immunoprecipitation of CD26 with CD45

The immunoprecipitation experiments described below provide evidence of a direct association between CD26 and CD45. Peripheral blood T cells ( $50 \times 10^6$ ) were labeled at the surface by lactoperoxidase-catalyzed iodination and immunoprecipitated from NP-40 lysis buffer (0.5% NP-40, 140mM NaCl, 1mM PMSF, 5mM EDTA, 50mM Tris HCl [pH 7.4]) or digitonin lysis buffer (1% digitonin, 0.12% Triton X-100, 150mM NaCl, 1mM PMSF, 20mM Triethanolamine [pH 7.8]) using anti-CD26 (Ta1, Coulter Immunology, Hialeah, FL; or 1F7, Dr. C. Morimoto, Dana-Farber Cancer Institute, Boston, MA) and anti-CD45 (GAP 8.3, Berger et al., *Human Immunol.* 3:231, 1981) as previously described by Morimoto et al. (*J. Immunol.* 143:3430, 1989) and Anderson et al. (*Nature* 341:159, 1989). All samples were analyzed under reducing conditions.

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For immunodepletion studies, peripheral blood T cells were labeled and lysed in digitonin lysis buffer as described above. The lysates were precleared by four successive immunoprecipitations with anti-CD45 (GAP 8.3, American Type Culture Collection, Bethesda, MD) or anti-CD1 (T6) and then precipitated by anti-CD26 and anti-CD45.

Digestion with V8 protease from *S. aureus* was carried out during gel electrophoresis as described by Cleveland et al. (*J. Biol. Chem.* 252:1102, 1977). After the first gel electrophoresis, gel slices containing the high molecular weight proteins co-precipitated with CD26 and CD45 proteins were excised and polymerized into the stacking gel of a 15% SDS-polyacrylamide gel. 2.5  $\mu$ g of V8 protease in 10  $\mu$ l of sample buffer (0.1% SDS, 0.125M Tris-HCl [pH 6.8], 10% glycerol, 0.1% bromophenol blue) were added to wells above the polymerized gel slices. Gel electrophoresis was carried out uninterrupted for 12 hours.

Fig. 8 presents the results of immunoprecipitation analysis without prior depletion. Surface labeled T-lymphocytes were solubilized in NP-40 (lanes 1-4) or digitonin (lanes 5-8) and immunoprecipitated with anti-CD1 (T6) as a negative control (lanes 1 and 5); anti-CD26 (1F7, lanes 2 and 6); anti-CD26 (Ta1, lanes 3 and 7); or anti-CD45 (GAP 8.3, lanes 4 and 8).

While anti-CD26 (Ta1 and 1F7) antibodies precipitated a 110KD molecule from NP-40 lysates under reducing conditions, in digitonin lysates these same antibodies precipitated two major proteins at 180 and 190KD and minor bands at 205 and 220kD in addition to the 110KD band. These additional bands display similar mobility to the CD45 control immunoprecipitates. In this regard, utilizing digitonin lysates or chemical cross-linkers, others have found an association of CD45 with

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Thy-1, CD3, and CD2 (Volarevic et al., *Proc. Natl. Acad. Sci. USA* 87:7085, 1990; Schraven et al., *Nature* 345:71, 1990).

To provide further evidence that the high molecular weight structure which co-precipitated with CD26 is CD45, we carried out both sequential immunodepletion and one-dimensional peptide mapping studies using V8 protease.

Fig. 9 presents the results of immunoprecipitation analysis of samples previously depleted for CD45 using anti-CD45 antibody (GAP 8.3, lanes 4-6) or, as a control, CD-1 using anti-CD1 antibody (T6, lanes 1-3). After depletion, anti-CD26 (1F7, lanes 1 and 4), anti-CD26 (Ta1, lanes 2 and 5), or anti-CD45 (GAP 8.3, lanes 3 and 6) was used for immunoprecipitation. As can be seen in Fig. 9, depletion of CD45 resulted in a complete loss of the high molecular weight structures in the CD26 immunoprecipitate (lanes 4, 5). Furthermore, V8 protease-dependent digestion of the high molecular weight molecules co-precipitated with either CD26 and CD45 yielded identical peptide patterns (Fig. 10). Although CD26 comodulated only with CD45RO (the 180kD isoform), the immunoprecipitation experiments suggest that CD26 is also associated with the 190kD isoform of CD45, and to a lesser degree, with the 205 and 220kD isoforms as well. These observations are consistent with earlier studies demonstrating that CD26 was preferentially expressed on CD45RO+ helper T cells, which are known to preferentially express both the 180 and 190kD isoforms of CD45 (Morimoto et al., *J. Immunol.* 143:3430, 1989; Rudd et al., *J. Exp. Med.* 166:1758, 1987; Terry et al., *Immunology* 64:331, 1988).

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Enhancement of the Kinase Activity of p56<sup>lck</sup> following anti-CD26 (1F7) Treatment

Recent studies have demonstrated that the cytoplasmic domain of CD45 has PTPase activity which regulates T cell activation pathways through dephosphorylation of phosphotyrosine (Charboneau et al., *Proc. Natl. Acad. Sci. USA* 85:7182, 1988; Ledbetter et al., *Proc. Natl. Acad. Sci., USA* 85:8628; Pingel et al., *Cell* 58:1055, 1989; Koretzky et al., *Nature* 346:66, 1990). One of the potential substrates for the CD45 PTPase is the tyrosine kinase p56<sup>lck</sup> (Osergaard et al., *Proc. Natl. Acad. Sci. USA* 86:8959, 1989; Mustelin et al., *Proc. Natl. Acad. Sci. USA* 86:6302, 1989), which itself may be involved in the CD3 chain phosphorylation (Veillette et al., *Nature* 338:257, 1989). CD26 may function in this system by enhancing CD3 phosphorylation through its association with CD45. If this model is correct, incubation with anti-CD26 (1F7) should alter p56<sup>lck</sup> kinase activity as measured by *in vitro* autophosphorylation.

To analyze *in vitro* kinase activity, samples of  $3.0 \times 10^7$  T lymphocytes were incubated in culture media with anti-CD26 (1F7) for various periods of time at 37°C. Immunoprecipitation and kinase analysis was then carried out as described by Rudal et al. (*Proc. Natl. Acad. Sci. USA* 85:5190, 1988). Cells were then solubilized in lysis buffer (1% NP-40, 20 mM TRIS-HCl [pH 8.0], 150 mM NaCl, 0.4 mM sodium vanadate, 0.5 mM EDTA, 10 mM NaF, 10 mM sodium pyrophosphate, 1 mM PMSF) for 30 min at 4°C. CD4 was immunoprecipitated from lysates containing equivalent amounts of total protein (500 µg) by a combination of anti-CD4 (19thy5D7; IgG2) and protein A-Sepharose. The immunoprecipitates were then washed extensively with lysis buffer prior to incubation with 30 µl of 25 mM Hepes containing 0.1% NP-40, and 10 µCi of [ $\lambda$ -<sup>32</sup>P]ATP (ICN,

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Costa Mesa, CA). After incubation of 15-30 min at 25°C, the reaction was stopped by the addition of sample buffer and the reaction products were resolved on 9% SDS-PAGE.

As shown in Fig. 11, the PTK activity of p56<sup>lck</sup> precipitated with CD4 significantly increased after 1 hour of incubation with anti-CD26 (lane 2) compared to a no-anti-CD26 control (lane 1). The kinase activity was even higher after 2, 3 or 4 hours of incubation with anti-CD26 (lanes 3-6, respectively). Concomitantly, the expression of CD26 on T cells treated with anti-CD26 (1F7) began to decrease within 1 hour of incubation and continued to decline as previously described (Dang et al., *J. Immunol.* 145:3936, 1990). Similar results were obtained when another anti-CD26 (Ta1) antibody was used. Nevertheless, incubation of cells with control anti-Class I MHC or anti-VLA 4 mAbs did not alter p56<sup>lck</sup> activity. The above results support the notion that the interaction of CD26 with CD45 enhances p56<sup>lck</sup> activity.

The kinetics of p56<sup>lck</sup> PTK activity (Fig. 11) and tyrosine phosphorylation of CD3 (Fig. 6) showed a similar pattern. This similarity supports the conclusion that tyrosine phosphorylation of CD3 induced by anti-CD26 is related to the PTK activity of p56<sup>lck</sup>. In addition, the similar kinetics also showed that the increase in p56<sup>lck</sup> PTK activity quickly affects the phosphorylation of CD3, as reported previously (Veilleux et al., *supra*). While the peak of the p56<sup>lck</sup> PTK activity or phosphorylation of CD3 induced by various stimuli is observed within minutes (Vivier et al., *supra*; Veillette et al., *supra*), the peak of either p56<sup>lck</sup> or CD3 phosphorylation induced by anti-CD26 treatment was observed after hours. In this regard, although the close relationship between CD45 PTPase activity and p56<sup>lck</sup> PTK activity has been reported (Ostergaard et al., *supra*; Mustelin et al., *supra*; Veillette et al., *supra*), the regulation of PTPase

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activity of CD45 has not yet been established. Therefore, it is possible that the change in PTPase activity or the interaction between CD45 PTPase and p56<sup>lck</sup> may require a relatively long time period following anti-  
5 CD26 treatment. It is also possible that the interaction between CD45 PTPase and p56<sup>lck</sup> is via an indirect rather than a direct mechanism.

CD26 is broadly distributed on non-hematopoietic cells. However, since the expression of CD45 is largely  
10 restricted to leukocytes, the association between CD26 and CD45 is probably found only on leukocytes. On the other hand, membrane-linked PTPases such as CD45 have been found on non-hematopoietic cells (Streuli et al., *J. Exp. Med.* 168:1553, 1988; Streuli et al., *Proc. Natl.*  
15 *Acad. Sci. USA* 86:8698, 1989; Lau et al. *Biochem J.* 257:23, 1989). Although the functional role of CD26 on nonhematopoietic cells is unclear, it is possible that CD26 is associated with the membrane-linked PTPase on nonhematopoietic cells.

20 In summary, we have demonstrated that anti-CD26-induced modulation resulted in enhanced CD3 phosphorylation and increased p56<sup>lck</sup> PTK activity. Both observations are consistent with the enhanced proliferative response of T cells following CD26  
25 modulation. These observations further suggest that the physical association of CD26 with CD45 may be key for CD26-mediated T cell signaling pathways. CD26 is known to be the membrane-associated ectoenzyme DPPIV which can cleave N-terminal dipeptides from polypeptides with  
30 either L-proline or L-alanine at the penultimate position. Although the natural ligand for CD26/DPPIV has not yet been established, binding of the natural substrate to the DPPIV enzyme may lead to cleavage and alteration in the biologic activity of the ligand. In  
35 light of the close proximity of the CD26 and CD45

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molecules, it is possible that CD26 modulates the enzymatic activity of the CD45 PTPase or perhaps affects the accessibility of critical substrates. This process would then enhance T cell activation via the CD3 or CD2  
5 pathway and could amplify the immune response *in vivo*. It should also be noted that increased numbers of CD26+ T lymphocytes have been found in both inflamed tissues and peripheral blood of patients with multiple sclerosis, Graves' Disease and rheumatoid arthritis (Hafler et al.,  
10 *N. Engl. J. Med.* 312:1405, 1985; Nakao et al., *J. Rheumatol.* 16:904, 1989; Eguchi et al., *J. Immunol.* 142:4233, 1989), suggesting that these CD26+ T cells may play an important role in chronic inflammation and in subsequent tissue damage.

#### 15 Soluble CD26 Fragments

Soluble fragments of CD26 are useful for interfering with CD26 activity. The fact that CD26 is a type II membrane protein suggests certain strategies for designing soluble fragments. For type II membrane  
20 proteins, the signal sequence used to transfer the protein across a membrane also serves as an anchor to the membrane. The cleavage of the signal sequence after protein transfer which usually occurs for other secreted proteins does not occur in type II transmembrane  
25 proteins. Thus, soluble forms of CD26 can be prepared by making its signal/anchor sequence accessible to a cellular proteolytic cleavage system. To accomplish this, the putative signal sequence of CD26 was shortened, as described below, since the 23 amino acid CD26 signal  
30 sequence is longer than most natural occurring cleavable signal sequences (von Heijne et al., *J. Mol. Biol.* 184:99, 1985). This is expected to result in proteolytic cleavage of the expressed polypeptide at or near one of the residues Ala Thr Ala corresponding to positions 35-37  
35 of wild type CD26, yielding a soluble fragment of CD26

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having at its amino terminus Ala<sub>35</sub>, Thr<sub>36</sub>, Ala<sub>37</sub> or Asp<sub>38</sub> of wild type CD26.

A first soluble CD26 construct is created by deleting the codons corresponding to amino acids 3-9 of intact CD26 (shown as the boxed amino acids in Fig. 13). The amino terminal sequence of the expressed polypeptide is MKGLLG-- (SEQ ID NO: 4) rather than the original MKTPWKVLLGLLG-- (SEQ ID NO: 5), and the potential proteolytic cleavage sites are shown as arrows in Fig. 13. This deletion mutant is prepared by oligonucleotide directed mutagenesis (see below) using the following oligonucleotide:

5'-ACGCCGACGATGAAGGGACTGCTGGGTGCT-3' (SEQ ID NO: 6).

A second construct is generated by taking advantage of the following rules proposed for signal peptide cleavage: (1) the residue in position -1 must be small, i.e., either Ala, Ser, Gly, Thr, Cys, Gln; (2) the residue in position -3 must not be aromatic (Phe, His, Tyr, Trp), charged (Asp, Glu, Lys, Arg), or large and polar (Asn, Gln); and (3) Pro must not be present at positions -3 through -1 (von Heijne, *Nuc. Acids Res.* 14:4683, 1986). Following these rules, we have designed a CD26 cDNA construct lacking codons corresponding to amino acids 24 to 34 of wild type CD26 (illustrated as the boxed amino acids in Fig. 14). This deletion mutant encodes the amino acid sequence

--IITVATADSR-- (SEQ ID NO: 7) instead of the original

--IITVPVLLNKGTDDATADSR-- (SEQ ID NO: 8), and the

potential proteolytic cleavage sites are shown as arrows in Fig. 14. This mutant is prepared by oligonucleotide-directed mutagenesis (see below) using the following oligonucleotide:

5'-ACCATCATCACCGTGGCTACAGCTGACAGT-3' (SEQ ID NO: 9). Site-directed mutagenesis is

performed as follows. The 3.0 kb CD26 cDNA fragment

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generated by the *Xba*I treatment of the original plasmid CDM7-CD26 is inserted into the *Xba*I site of pTZ19u (Bio-rad). A recombinant plasmid which inserts the cDNA inverse to the *lacZ* gene on the plasmid is identified by  
5 restriction enzyme mapping and used for subsequent mutagenesis.

Using single-stranded DNA prepared from this plasmid as a template and the previously-described oligonucleotides as primers, oligonucleotide-directed  
10 mutagenesis is performed by the method of Kunkel (*Proc. Natl. Acad. Sci. USA* 82:488, 1985), using a commercially available kit (BioRad, Richmond, CA).

To obtain high level expression of soluble CD26, a new expression vector is constructed. First the *Xba*I  
15 CD26 cDNA fragment of pTZ19u-CD26 and the *Hind*III-*Xba*I vector fragment of Rc/CMV (Invitrogene, San Diego, CA) are treated with Klenow enzyme and ligated. The resulting plasmid is screened by restriction enzyme mapping for the insertion of the CD26 cDNA fragment under  
20 the control of the CMV promoter. This construct leaves one *Xba*I site just in front of the CD26 cDNA. Then, the *Mlu*I-*Xba*I CMV promoter DNA fragment of this plasmid DNA is exchanged with the *Hind*III-*Xba*I SR $\alpha$  promoter DNA fragment of pSR $\alpha$ -26 to give a final expression vector  
25 RcSR $\alpha$ -26. Next, the above mutant CD26 cDNAs are transferred to this expression vector. The *Xba*I-*Dra*III DNA fragment derived from the mutant cDNAs which encoded the mutant part and the wild type 2.0 kb *Dra*III-*Hind*III DNA fragment are ligated with the *Xba*I-*Hind*III vector  
30 fragment of RcSR $\alpha$ -26. The expression plasmid which has the  $\Delta$ 3-9 or  $\Delta$ 24-34 mutant CD26 cDNA is identified by restriction enzyme mapping and DNA sequencing. The resultant plasmids RcSR $\alpha$ -26. $\Delta$ 3-9 and RcSR $\alpha$ -26. $\Delta$ 24-34 are used to transfect Jurkat cells or CHO cells.

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Jurkat cells are transfected with these plasmids as described above except pSVneo-sp is omitted from the donor DNA mixture since the RCSR $\alpha$  plasmid already carries the neo resistance marker. Neo-resistant clones are  
5 screened by metabolic labelling and immunoprecipitation (Harlow et al., eds. *Antibodies: a laboratory manual*, Cold Spring Harbor Laboratory, 1988) for the expression of soluble CD26. The transfectants which produce a large amount of soluble CD26 are used for protein production.

10 CHO cells transfected with the DNA mixture of pMT2 and RCSR $\alpha$ -26. $\Delta$ 3-9 or RCSR $\alpha$ -26. $\Delta$ 24-34 are selected for their growing ability in  $\alpha$ -medium and the production of soluble CD26. The expression of the soluble protein is amplified by culturing the transfected CHO cells in  
15 medium containing an increasing amount of MTX. Although both Jurkat cells and CHO cells can provide the soluble form of CD26, the protein produced by Jurkat cells is preferred because of its human T cell origin.

Another approach to making fragments of CD26 is  
20 illustrated by the following:

Ligation of the CD26 XbaI-SphI cDNA fragment to the vector RCSR $\alpha$ -26 XbaI-HindIII DNA fragment and the following synthetic DNA linker:

5'-----CATAGTAATCGATA

25 GTACGTATCATTAGCTATTCTGA-----5' (SEQ ID NO: 10) introduces an in-frame stop codon that results in deletion of the segment of CD26 from amino acid 594 to the carboxy terminus of the wild-type protein. This deletion mutant, which is shown in Fig. 15 (SEQ ID NO:  
30 11), lacks the putative catalytic site of CD26 and has a new carboxy terminus of --GDKIMHA (SEQ ID NO: 12).

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CD26 Derivatives Capable of Disrupting CD26/CD45 Interaction

Other polypeptide fragments of CD26 can be produced by standard methods of protein synthetic chemistry, using the information disclosed herein to design appropriate polypeptides and assay them for biological activity. A preferred method of producing such fragments, however, is by the use of recombinant DNA techniques. For example, the sequence of CD26 given in Fig. 1 (SEQ ID NO:1) can be used to design oligonucleotides encoding fragments of CD26 containing deletions of nonessential CD26 amino acid residues from the beginning, the end, and/or any central portion of the protein; such oligonucleotides are chemically synthesized by known methods and inserted into expression vectors for expression of a polypeptide fragment of CD26. Alternatively, one may manipulate the CD26 coding regions of CD26 expression plasmids by site-directed mutagenesis, as disclosed above for two such fragments of CD26, or by insertion of a stop codon at an appropriate place in the coding sequence. The CD26 fragment can then be produced in transfected cultured cells in large quantities, purified by standard methods, and tested in an assay such as the immunoprecipitation assay described above, which is useful for identifying fragments capable of disrupting the interaction of CD26 and CD45. Briefly, surface-labeled peripheral blood T cells which express both CD26 and CD45 (or any mammalian cells transfected with cDNAs encoding CD26 and CD45 so that both proteins are functionally expressed on the cells' surfaces) are incubated in the presence and absence of a CD26 polypeptide fragment. The cells are lysed in digitonin lysis buffer, and anti-CD45 monoclonal antibody is used to immunoprecipitate CD45 and any proteins associated with CD45. The amount of CD26 that co-precipitates with

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CD45 in the presence of a given polypeptide fragment can be determined by known methods (e.g., by densitometer readings of the labelled bands on an SDS-PAGE gel analyzing the constituents of an immunoprecipitate) and compared to the amount that co-precipitates with CD45 in the absence of the polypeptide fragment. Alternatively, one can instead use an anti-CD26 antibody and measure the relative amounts of CD45 that co-precipitate with CD26 in the presence and absence of the given polypeptide fragment. If an anti-CD26 antibody is used, it is preferred that the antibody does not substantially bind to the competitor CD26 polypeptide; such binding interferes with the assay. In either case, CD26 polypeptide fragments which interfere with the interaction between CD26 and CD45 will decrease co-precipitation.

An analysis similar to that described above can be used to identify polypeptide fragments of CD45 which disrupt CD26/CD45 interaction. When screening CD45 fragments, it is preferable to perform the immunoprecipitation with anti-CD26 antibody.

#### Association of p43 with CD26

When CD26 is immunoprecipitated from surface-labelled T cells and the immunoprecipitate is analyzed on SDS-PAGE, two bands are typically seen: one at 110kDa, corresponding to CD26, and a second, much fainter band at 43kDa. This lower molecular weight protein is termed "p43". Fig. 12 illustrates one such experiment, in which E+ cells were labeled by lactoperoxidase-catalyzed iodination and lysed in NP-40 lysis buffer for immunoprecipitation as described above. Precipitates were analyzed by 9% SDS-PAGE. Lane 1: anti-CD1 (T6) as negative control; lane 2: anti-1F7; lane 3: anti-Ta1; lane 4: anti-5F8 (another anti-CD26 monoclonal antibody);

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lane 5: anti-CD29 (4B4) as control. As shown in Fig. 12, anti-1F7 brought down an obvious 43kDa structure (lane 2) from surface-labeled T cells. On the other hand, this structure was detected faintly following anti-Ta1 or anti-5F8 precipitation (lanes 3 and 4). This band was not detected following anti-CD1 or anti-CD29 precipitation (lanes 1 and 5). Similar results were seen when the cells were human thymocytes or from the human T cell lines H9 or Peer IV (data not shown). In other anti-Ta1 or anti-5F8 immunoprecipitation experiments using T cells from other donors, the 43kDa band was sometimes more distinct than those shown in lanes 3 and 4 of Fig. 12. In addition, a third band at approximately 70 kDa is sometimes observed in these CD26 immunoprecipitation experiments. Because they are found in association with the 110 kDa CD26 molecule, both the 43 kDa molecule and the 70 kDa molecule may play important roles in T cell activation. Compounds (such as fragments or analogs of CD26) which interfere with the association of CD26 with either p43 or the 70 kDa molecule may be detected by means of a screening assay patterned on those described above with respect to CD26 and CD45.

It is thought to be unlikely that anti-1F7 cross-reacts with p43, since the density of the 43kDa band decreased after repeated preclearing by either anti-Ta1 or anti-5F8. Although the reasons for the variability in the detection of p43 are not clear, it is possible that the binding of anti-CD26 mAbs may generate conformational changes in CD26, affecting the association of the 43 kDa molecule with the 110 kDa molecule. It is also possible that the Ta1 or 5F8 epitope may be close to the association site between the 43 and 110 kDa molecules, such that binding of these mAbs may inhibit the association of these molecules with each other.

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P43 may be purified by affinity chromatography, using an anti-CD26 monoclonal antibody to purify the CD26-p43 complex from T cell membranes. P43 may then be separated from CD26 by SDS-PAGE, followed by HPLC if  
5 further purification is necessary. Affinity chromatography with monoclonal antibodies, SDS-PAGE, and HPLC are all standard methods well known to those of ordinary skill in the art.

Hybridization probes based upon a partial amino  
10 acid sequence of the purified protein may be used to select p43 cDNA from a T cell library. Alternatively, the partial amino acid sequence can be used to design PCR primers for priming synthesis of a partial p43 cDNA on mRNA templates, using standard methods, and the resulting  
15 partial cDNA used as a probe to detect full-length p43 cDNA in a T cell library. This cDNA can be inserted in an expression plasmid and used to transfect cells which do not naturally express the p43 gene. Such cells would be useful for use as an antigen to develop anti-p43  
20 monoclonal antibodies, and also as a means to study the role of p43 in T cell activation. They can also be used in the screening assay referred to above.

#### Northern Analysis Using a CD26 cDNA Probe

Analysis of the degree of expression of CD26 in  
25 any given cell type or tissue type can be accomplished using the standard technique of Northern blotting, probing with a labelled, single stranded nucleic acid molecule derived from the coding region of CD26 cDNA. The probe would have a sequence based upon the sense  
30 strand of SEQ ID NO: 1, which is complementary to CD26 mRNA, and preferably would be at least 8 nucleotides in length (more preferably at least 14 nucleotides, and most preferably at least 30). The probe may contain most or all of the entire coding sequence of CD26 cDNA. Such an  
35 assay, which would be useful for diagnosing conditions

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characterized by the over- or under-expression of CD26 in a given cell type, such as T cells, would include the following steps:

- 5 (a) providing a biological sample containing mRNA of a cell;
- (b) contacting the sample with a single-stranded nucleic acid probe as described above; and
- (c) detecting hybridization of the probe with the sample, which hybridization would be indicative of the  
10 presence of CD26 mRNA in the cell.

Other embodiments are within the following claims.

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SEQUENCE LISTING

## (1) GENERAL INFORMATION:

(i) APPLICANT: Dana-Farber Cancer Institute,  
Inc.

(ii) TITLE OF INVENTION: HUMAN CD26 AND METHODS FOR USE

(iii) NUMBER OF SEQUENCES: 12

(iv) CORRESPONDENCE ADDRESS:

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(B) STREET: 225 Franklin Street  
(C) CITY: Boston  
(D) STATE: Massachusetts  
(E) COUNTRY: U.S.A.  
(F) ZIP: 02110-2804

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5" Diskette, 1.44 Mb  
(B) COMPUTER: IBM PS/2 Model 50Z or 55SX  
(C) OPERATING SYSTEM: IBM P.C. DOS (Version 3.30)  
(D) SOFTWARE: WordPerfect (Version 5.0)

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:  
(B) FILING DATE:  
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 07/832,211  
(B) FILING DATE: February 6, 1992

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(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 1:

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## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	2924
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	double
(D) TOPOLOGY:	linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GACGCCGACG	ATG	AAG	ACA	CCG	TGG	AAG	GTT	CTT	CTG	GGA	CTG	CTG	GGT	49
	Met	Lys	Thr	Pro	Trp	Lys	Val	Leu	Leu	Gly	Leu	Leu	Gly	
	1				5					10				
GCT GCT GCG CTT GTC ACC ATC ATC ACC GTG CCC GTG GTT CTG CTG AAC	97													
Ala Ala Ala Leu Val Thr Ile Ile Thr Val Pro Val Val Leu Leu Asn														
15 20 25														
AAA GGC ACA GAT GAT GCT ACA GCT GAC AGT CGC AAA ACT TAC ACT CTA	145													
Lys Gly Thr Asp Asp Ala Thr Ala Asp Ser Arg Lys Thr Tyr Thr Leu														
30 35 40 45														
ACT GAT TAC TTA AAA AAT ACT TAT AGA CTG AAG TTA TAC TCC TTA AGA	193													
Thr Asp Tyr Leu Lys Asn Thr Tyr Arg Leu Lys Leu Tyr Ser Leu Arg														
50 55 60														
TGG ATT TCA GAT CAT GAA TAT CTC TAC AAA CAA GAA AAT AAT ATC TTG	241													
Trp Ile Ser Asp His Glu Tyr Leu Tyr Lys Gln Glu Asn Asn Ile Leu														
65 70 75														
GTA TTC AAT GCT GAA TAT GGA AAC AGC TCA GTT TTC TTG GAG AAC AGT	289													
Val Phe Asn Ala Glu Tyr Gly Asn Ser Ser Val Phe Leu Glu Asn Ser														
80 85 90														

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ACA TTT GAT GAG TTT GGA CAT TCT ATC AAT GAT TAT TCA ATA TCT CCT	337
Thr Phe Asp Glu Phe Gly His Ser Ile Asn Asp Tyr Ser Ile Ser Pro	
95 100 105	
GAT GGG CAG TTT ATT CTC TTA GAA TAC AAC TAC GTG AAG CAA TGG AGG	385
Asp Gly Gln Phe Ile Leu Leu Glu Tyr Asn Tyr Val Lys Gln Trp Arg	
110 115 120 125	
CAT TCC TAC ACA GCT TCA TAT GAC ATT TAT GAT TTA AAT AAA AGG CAG	433
His Ser Tyr Thr Ala Ser Tyr Asp Ile Tyr Asp Leu Asn Lys Arg Gln	
130 135 140	
CTG ATT ACA GAA GAG AGG ATT CCA AAC AAC ACA CAG TGG GTC ACA TGG	481
Leu Ile Thr Glu Glu Arg Ile Pro Asn Asn Thr Gln Trp Val Thr Trp	
145 150 155	
TCA CCA GTG GGT CAT AAA TTG GCA TAT GTT TGG AAC AAT GAC ATT TAT	529
Ser Pro Val Gly His Lys Leu Ala Tyr Val Trp Asn Asn Asp Ile Tyr	
160 165 170	
GTT AAA ATT GAA CCA AAT TTA CCA AGT TAC AGA ATC ACA TGG ACG GGG	577
Val Lys Ile Glu Pro Asn Leu Pro Ser Tyr Arg Ile Thr Trp Thr Gly	
175 180 185	
AAA GAA GAT ATA ATA TAT AAT GGA ATA ACT GAC TGG GTT TAT GAA GAG	625
Lys Glu Asp Ile Ile Tyr Asn Gly Ile Thr Asp Trp Val Tyr Glu Glu	
190 195 200 205	
GAA GTC TTC AGT GCC TAC TCT GCT CTG TGG TGG TCT CCA AAC GGC ACT	673
Glu Val Phe Ser Ala Tyr Ser Ala Leu Trp Trp Ser Pro Asn Thr Thr	
210 215 220	
TTT TTA GCA TAT GCC CAA TTT AAC GAC ACA GAA GTC CCA CTT ATT GAA	721
Phe Leu Ala Tyr Ala Gln Phe Asn Asp Thr Glu Val Pro Leu Ile Glu	
225 230 235	
TAC TCC TTC TAC TCT GAT GAG TCA CTG CAG TAC CCA AAG ACT GTA CGG	769
Tyr Ser Phe Tyr Ser Asp Glu Ser Leu Gln Tyr Pro Lys Thr Val Arg	
240 245 250	
GTT CCA TAT CCA AAG GCA GGA GCT GTG AAT CCA ACT GTA AAG TTC TTT	817
Val Pro Tyr Pro Lys Ala Gly Ala Val Asn Pro Thr Val Lys Phe Phe	
255 260 265	
GTT GTA AAT ACA GAC TCT CTC AGC TCA GTC ACC AAT GCA ACT TCC ATA	865
Val Val Asn Thr Asp Ser Leu Ser Ser Val Thr Asn Ala Thr Ser Ile	
270 275 280 285	
CAA ATC ACT GCT CCT GCT TCT ATG TTG ATA GGG GAT CAC TAC TTG TGT	913
Gln Ile Thr Ala Pro Ala Ser Met Leu Ile Gly Asp His Tyr Leu Cys	
290 295 300	

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GAT GTG ACA TGG GCA ACA CAA GAA AGA ATT TCT TTG CAG TGG CTC AGG	961
Asp Val Thr Trp Ala Thr Gln Glu Arg Ile Ser Leu Gln Trp Leu Arg	
305 310 315	
AGG ATT CAG AAC TAT TCG GTC ATG GAT ATT TGT GAC TAT GAT GAA TCC	1009
Arg Ile Gln Asn Tyr Ser Val Met Asp Ile Cys Asp Tyr Asp Glu Ser	
320 325 330	
AGT GGA AGA TGG AAC TGC TTA GTG GCA CGG CAA CAC ATT GAA ATG AGT	1057
Ser Gly Arg Trp Asn Cys Leu Val Ala Arg Gln His Ile Glu Met Ser	
335 340 345	
ACT ACT GGC TGG GTT GGA AGA TTT AGG CCT TCA GAA CCT CAT TTT ACC	1105
Thr Thr Gly Trp Val Gly Arg Phe Arg Pro Ser Glu Pro His Phe Thr	
350 355 360 365	
CTT GAT GGT AAT AGC TTC TAC AAG ATC ATC AGC AAT GAA GAA GGT TAC	1153
Leu Asp Gly Asn Ser Phe Tyr Lys Ile Ile Ser Asn Glu Glu Gly Tyr	
370 375 380	
AGA CAC ATT TGC TAT TTC CAA ATA GAT AAA AAA GAC TGC ACA TTT ATT	1201
Arg His Ile Cys Tyr Phe Gln Ile Asp Lys Lys Asp Cys Thr Phe Ile	
385 390 395	
ACA AAA GGC ACC TGG GAA GTC ATC GGG ATA GAA GCT CTA ACC AGT GAT	1249
Thr Lys Gly Thr Trp Glu Val Ile Gly Ile Glu Ala Leu Thr Ser Asp	
400 405 410	
TAT CTA TAC TAC ATT AGT AAT GAA TAT AAA GGA ATG CCA GGA GGA AGG	1297
Tyr Leu Tyr Tyr Ile Ser Asn Glu Tyr Lys Gly Met Pro Gly Gly Arg	
415 420 425	
AAT CTT TAT AAA ATC CAA CTT AGT GAC TAT ACA AAA GTG ACA TGC CTC	1345
Asn Leu Tyr Lys Ile Gln Leu Ser Asp Tyr Thr Lys Val Thr Cys Leu	
430 435 440 445	
AGT TGT GAG CTG AAT CCG GAA AGG TGT CAG TAC TAT TCT GTG TCA TTC	1393
Ser Cys Glu Leu Asn Pro Glu Arg Cys Gln Tyr Tyr Ser Val Ser Phe	
450 455 460	
AGT AAA GAG GCG AAG TAT TAT CAG CTG AGA TGT TCC GGT CCT GGT CTG	1441
Ser Lys Glu Ala Lys Tyr Tyr Gln Leu Arg Cys Ser Gly Pro Gly Leu	
465 470 475	
CCC CTC TAT ACT CTA CAC AGC AGC GTG AAT GAT AAA GGG CTG AGA GTC	1489
Pro Leu Tyr Thr Leu His Ser Ser Val Asn Asp Lys Gly Leu Arg Val	
480 485 490	
CTG GAA GAC AAT TCA GCT TTG GAT AAA ATG CTG CAG AAT GTC CAG ATG	1537
Leu Glu Asp Asn Ser Ala Leu Asp Lys Met Leu Gln Asn Val Gln Met	
495 500 505	

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CCC TCC AAA AAA CTG GAC TTC ATT ATT TTG AAT GAA ACA AAA TTT TGG	1585
Pro Ser Lys Lys Leu Asp Phe Ile Ile Leu Asn Glu Thr Lys Phe Trp	
510 515 520 525	
TAT CAG ATG ATC TTG CCT CCT CAT TTT GAT AAA TCC AAG AAA TAT CCT	1633
Tyr Gln Met Ile Leu Pro Pro His Phe Asp Lys Ser Lys Lys Tyr Pro	
530 535 540	
CTA CTA TTA GAT GTG TAT GCA GGC CCA TGT AGT CAA AAA GCA GAC ACT	1681
Leu Leu Leu Asp Val Tyr Ala Gly Pro Cys Ser Gln Lys Ala Asp Thr	
545 550 555	
GTC TTC AGA CTG AAC TGG GCC ACT TAC CTT GCA AGC ACA GAA AAC ATT	1729
Val Phe Arg Leu Asn Trp Ala Thr Tyr Leu Ala Ser Thr Glu Asn Ile	
560 565 570	
ATA GTA GCT AGC TTT GAT GGC AGA GGA AGT GGT TAC CAA GGA GAT AAG	1777
Ile Val Ala Ser Phe Asp Gly Arg Gly Ser Gly Tyr Gln Gly Asp Lys	
575 580 585	
ATC ATG CAT GCA ATC AAC AGA AGA CTG GGA ACA TTT GAA GTT GAA GAT	1825
Ile Met His Ala Ile Asn Arg Arg Leu Gly Thr Phe Glu Val Glu Asp	
590 595 600 605	
CAA ATT GAA GCA GCC AGA CAA TTT TCA AAA ATG GGA TTT GTG GAC AAC	1873
Gln Ile Glu Ala Ala Arg Gln Phe Ser Lys Met Gly Phe Val Asp Asn	
610 615 620	
AAA CGA ATT GCA ATT TGG GGC TGG TCA TAT GGA GGG TAC GTA ACC TCA	1921
Lys Arg Ile Ala Ile Trp Gly Trp Ser Tyr Gly Gly Tyr Val Thr Ser	
625 630 635	
ATG GTC CTG GGA TCA GGA AGT GGC GTG TTC AAG TGT GGA ATA GCC GTG	1969
Met Val Leu Gly Ser Gly Ser Gly Val Phe Lys Cys Gly Ile Ala Val	
640 645 650	
GCG CCT GTA TCC CGG TGG GAG TAC TAT GAC TCA GTG TAC ACA GAA CGT	2017
Ala Pro Val Ser Arg Trp Glu Tyr Tyr Asp Ser Val Tyr Thr Glu Arg	
655 660 665	
TAC ATG GGT CTC CCA ACT CCA GAA GAC AAC CTT GAC CAT TAC AGA AAT	2065
Tyr Met Gly Leu Pro Thr Pro Glu Asp Asn Leu Asp His Tyr Arg Asn	
670 675 680 685	
TCA ACA GTC ATG AGC AGA GCT GAA AAT TTT AAA CAA GTT GAG TAC CTC	2113
Ser Thr Val Met Ser Arg Ala Glu Asn Phe Lys Gln Val Glu Tyr Leu	
690 695 700	
CTT ATT CAT GGA ACA GCA GAT GAT AAC GTT CAC TTT CAG CAG TCA GCT	2161
Leu Ile His Gly Thr Ala Asp Asp Asn Val His Phe Gln Gln Ser Ala	
705 710 715	

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CAG ATC TCC AAA GCC CTG GTC GAT GTT GGA GTG GAT TTC CAG GCA ATG 2209  
 Gln Ile Ser Lys Ala Leu Val Asp Val Gly Val Asp Phe Gln Ala Met  
 720 725 730

TGG TAT ACT GAT GAA GAC CAT GGA ATA GCT AGC AGC ACA GCA CAC CAA 2257  
 Trp Tyr Thr Asp Glu Asp His Gly Ile Ala Ser Ser Thr Ala His Gln  
 735 740 745

CAT ATA TAT ACC CAC ATG AGC CAC TTC ATA AAA CAA TGT TTC TCT TTA 2305  
 His Ile Tyr Thr His Met Ser His Phe Ile Lys Gln Cys Phe Ser Leu  
 750 755 760 765

CCT TAGCACCTCA AAATACCATG CCATTAAAG CTTATTAAAA CTCATTTTTC 2358  
 Pro

TTTTCATTAT CTCAAAACCTG CACTGTCAAG ATGATGATGA TCTTTAAAAT ACACACTCAA 2418

ATCAAGAAAC TTAAGGTAC CTTTGTCCC AAATTCATA CCTATCATCT TAAGTAGGGA 2478

CTTCTGTCTT CACAACAGAT TATTACCTTA CAGAAGTTTG AATTATCCGG TCGGGTTTTTA 2538

TTGTTTAAAA TCATTTCTGC ATCAGCTGCT GAAACAACAA ATAGGAATTG TTTTATGGA 2598

GGCTTTGCAT AGATTCCCTG AGCAGGATTT TAATCTTTTT CTAAGTGGAC TGGTTCAAAT 2658

GTTGTCTCT TCTTTAAAGG GATGGCAAGA TGTGGGCAGT GATGTCACTA GGGCAGGGAC 2718

AGGATAAGAG GGATTAGGGA GAGAAGATAG CAGGGCATGG CTGGGAACCC AAGTCCAAGC 2778

ATACCAACAC GACCAGGCTA CTGTCAGCTC CCCTCGGAGA AACTGTGCA GTCTGCGTGT 2838

GAACAGCTCT TCTCCTTTAG AGCACAATGG ATCTCGAGGG ATCTCCATA CCTACCAGTT 2898

CTGCGCCTCG AGGCCGCGAC TCTAGA 2924

## (2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Thr Pro Trp Lys Val Leu Ile  
 1 5

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## (2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 3:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Pro Val Val Leu Leu Asn Lys Gly Thr Asp Asp  
1 5 10

## (2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 4:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Lys Gly Leu Leu Gly  
1 5

## (2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 5:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Met Lys Thr Pro Trp Lys Val Leu Leu Gly Leu Leu Gly  
1 5 10

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## (2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 6:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

ACGCCGACGA TGAAGGGACT GCTGGGTGCT

30

## (2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 7:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Ile Ile Thr Val Ala Thr Ala Asp Ser Arg  
1 5 10

## (2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 8:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Ile Ile Thr Val Pro Val Val Leu Leu Asn Lys Gly Thr Asp Asp Ala  
1 5 10 15

Thr Ala Asp Ser Arg  
20

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## (2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 9:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

ACCATCATCA CCGTGGCTAC AGCTGACAGT

30

## (2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 10:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GTACGTATCA TTAGCTATTC GA

22

## (2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 11:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 603  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: N/A  
 (D) TOPOLOGY: N/A

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Met Lys Thr Pro Trp Lys Val Leu Leu Gly Leu Leu Gly Ala Ala Ala  
 1 5 10 15

Leu Val Thr Ile Ile Thr Val Pro Val Val Leu Leu Asn Lys Gly Thr  
 20 25 30

Asp Asp Ala Thr Ala Asp Ser Arg Lys Thr Tyr Thr Leu Thr Asp Tyr  
 35 40 45

Leu Lys Asn Thr Tyr Arg Leu Lys Leu Tyr Ser Leu Arg Trp Ile Ser  
 50 55 60

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Asp His Glu Tyr Leu Tyr Lys Gln Glu Asn Asn Ile Leu Val Phe Asn  
 65 70 75 80  
 Ala Glu Tyr Gly Asn Ser Ser Val Phe Leu Glu Asn Ser Thr Phe Asp  
 85 90 95  
 Glu Phe Gly His Ser Ile Asn Asp Tyr Ser Ile Ser Pro Asp Gly Gln  
 100 105 110  
 Phe Ile Leu Leu Glu Tyr Asn Tyr Val Lys Gln Trp Arg His Ser Tyr  
 115 120 125  
 Thr Ala Ser Tyr Asp Ile Tyr Asp Leu Asn Lys Arg Gln Leu Ile Thr  
 130 135 140  
 Glu Glu Arg Ile Pro Asn Asn Thr Gln Trp Val Thr Trp Ser Pro Val  
 145 150 155 160  
 Gly His Lys Leu Ala Tyr Val Trp Asn Asn Asp Ile Tyr Val Lys Ile  
 165 170 175  
 Glu Pro Asn Leu Pro Ser Tyr Arg Ile Thr Trp Thr Gly Lys Glu Asp  
 180 185 190  
 Ile Ile Tyr Asn Gly Ile Thr Asp Trp Val Tyr Glu Glu Glu Val Phe  
 195 200 205  
 Ser Ala Tyr Ser Ala Leu Trp Trp Ser Pro Asn Gly Thr Phe Leu Ala  
 210 215 220  
 Tyr Ala Gln Phe Asn Asp Thr Glu Val Pro Leu Ile Glu Tyr Ser Phe  
 225 230 235 240  
 Tyr Ser Asp Glu Ser Leu Gln Tyr Pro Lys Thr Val Arg Val Pro Tyr  
 245 250 255  
 Pro Lys Ala Gly Ala Val Asn Pro Thr Val Lys Phe Phe Val Val Asn  
 260 265 270  
 Thr Asp Ser Leu Ser Ser Val Thr Asn Ala Thr Ser Ile Gln Ile Thr  
 275 280 285  
 Ala Pro Ala Ser Met Leu Ile Gly Asp His Tyr Leu Cys Asp Val Thr  
 290 295 300  
 Trp Ala Thr Gln Glu Arg Ile Ser Leu Gln Trp Leu Arg Arg Ile Gln  
 305 310 315 320  
 Asn Tyr Ser Val Met Asp Ile Cys Asp Tyr Asp Glu Ser Ser Gly Arg  
 325 330 335 340

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Trp Asn Cys Leu Val Ala Arg Gln His Ile Glu Met Ser Thr Thr Gly  
 345 350 355  
 Trp Val Gly Arg Phe Arg Pro Ser Glu Pro His Phe Thr Leu Asp Gly  
 360 365 370  
 Asn Ser Phe Tyr Lys Ile Ile Ser Asn Glu Glu Gly Tyr Arg His Ile  
 375 380 385  
 Cys Tyr Phe Gln Ile Asp Lys Lys Asp Cys Thr Phe Ile Thr Lys Gly  
 390 395 400 405  
 Thr Trp Glu Val Ile Gly Ile Glu Ala Leu Thr Ser Asp Tyr Leu Tyr  
 410 415 420  
 Tyr Ile Ser Asn Glu Tyr Lys Gly Met Pro Gly Gly Arg Asn Leu Tyr  
 425 430 435  
 Lys Ile Gln Leu Ser Asp Tyr Thr Lys Val Thr Cys Leu Ser Cys Glu  
 440 445 450  
 Leu Asn Pro Glu Arg Cys Gln Tyr Tyr Ser Val Ser Phe Ser Lys Glu  
 455 460 465  
 Ala Lys Tyr Tyr Gln Leu Arg Cys Ser Gly Pro Gly Leu Pro Leu Tyr  
 470 475 480 485  
 Thr Leu His Ser Ser Val Asn Asp Lys Gly Leu Arg Val Leu Glu Asp  
 490 495 500  
 Asn Ser Ala Leu Asp Lys Met Leu Gln Asn Val Gln Met Pro Ser Lys  
 505 510 515  
 Lys Leu Asp Phe Ile Ile Leu Asn Glu Thr Lys Phe Trp Tyr Gln Met  
 520 525 530  
 Ile Leu Pro Pro His Phe Asp Lys Ser Lys Lys Tyr Pro Leu Leu Leu  
 535 540 545  
 Asp Val Tyr Ala Gly Pro Cys Ser Gln Lys Ala Asp Thr Val Phe Arg  
 550 555 560 565  
 Leu Asn Trp Ala Thr Tyr Leu Ala Ser Thr Glu Asn Ile Ile Val Ala  
 570 575 580 585  
 Ser Phe Asp Gly Arg Gly Ser Gly Tyr Gln Gly Asp Lys Ile Met His  
 590 595 600  
 Ala

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(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	7
(B) TYPE:	amino acid
(C) STRANDEDNESS:	
(D) TOPOLOGY:	

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Gly	Asp	Lys	Ile	Met	His	Ala
1				5		

What is claimed is:

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Claims

1. A polypeptide fragment of CD26 having an amino acid sequence substantially identical to the amino acid sequence of SEQ ID NO: 2 ( $\Delta$ 3-9).
- 5        2. A nucleic acid encoding the polypeptide of claim 1.
3. A polypeptide fragment of CD26 having an amino acid sequence substantially identical to the amino acid sequence of SEQ ID NO: 3 ( $\Delta$ 24-34).
- 10       4. A nucleic acid encoding the polypeptide of claim 3.
5. The polypeptide of claim 1, wherein said polypeptide has an amino acid sequence identical to the amino acid sequence of SEQ ID NO: 2.
- 15       6. The polypeptide of claim 3, wherein said polypeptide has an amino acid sequence identical to the amino acid sequence of SEQ ID NO: 3.
7. The polypeptide of claim 1, said polypeptide being soluble under physiological conditions.
- 20       8. The polypeptide of claim 3, said polypeptide being soluble under physiological conditions.
9. The polypeptide of claim 1, said polypeptide being substantially pure.
10. The polypeptide of claim 3, said polypeptide  
25 being substantially pure.

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11. A plasmid comprising the nucleic acid of any of claims 2 or 4.

12. The plasmid of claim 11, said plasmid further comprising an expression control sequence capable of  
5 directing expression of said polypeptide.

13. A polypeptide fragment of CD26 or analogs thereof capable of disrupting the naturally occurring binding interaction between CD45 and CD26.

14. A method for screening candidate compounds to  
10 identify compounds capable of inhibiting the binding of CD26 to CD45, said method comprising the steps of:

(a) providing a first and a second sample of cells expressing both CD26 and CD45;

(b) incubating said first sample in the presence  
15 of a candidate compound;

(c) incubating said second sample in the absence of said candidate compound;

(d) generating a first immunoprecipitate by adding to said first sample a first aliquot of an anti-CD26  
20 antibody;

(e) generating a second immunoprecipitate by adding to said second sample a second aliquot of said antibody; and

(f) determining whether the amount of CD45 present  
25 in said first immunoprecipitate is less than the amount of CD45 present in said second immunoprecipitate, the presence of a lesser amount of CD45 in said first immunoprecipitate than in said second immunoprecipitate indicating that said candidate compound inhibits said  
30 binding.

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15. A method for screening candidate compounds to identify compounds capable of inhibiting the binding of CD26 to CD45, said method comprising the steps of:

(a) providing a first and a second sample of cells  
5 expressing both CD26 and CD45;

(b) incubating said first sample in the presence of a candidate compound;

(c) incubating said second sample in the absence of said candidate compound;

10 (d) generating a first immunoprecipitate by adding to said first sample a first aliquot of an anti-CD45 antibody;

(e) generating a second immunoprecipitate by adding to said second sample a second aliquot of said  
15 antibody; and

(f) determining whether the amount of CD26 present in said first immunoprecipitate is less than the amount of CD26 present in said second immunoprecipitate, the presence of a lesser amount of CD26 in said first  
20 immunoprecipitate than in said second immunoprecipitate indicating that said candidate compound inhibits said binding.

16. A monoclonal antibody which, when contacted under physiological conditions with a cell expressing  
25 CD26 and CD45, interferes with the association of said CD26 and CD45.

17. A method comprising:

(a) providing a cell which expresses CD45 on its surface; and

30 (b) introducing into said cell a nucleic acid encoding CD26, such that said cell expresses CD26 on its surface.

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18. A method comprising:

(a) providing a cell which expresses CD26 on its surface; and

5 (b) introducing into said cell a nucleic acid encoding CD45, such that said cell expresses CD45 on its surface.

19. A cell transfected with a nucleic acid encoding CD26, said cell expressing both CD26 and CD45 on its surface.

10 20. A cell transfected with a nucleic acid encoding CD45, said cell expressing both CD26 and CD45 on its surface.

21. The cell of claim 19, wherein said cell is a Jurkat cell.

15 22. The cell of claim 20, wherein said cell is a Jurkat cell.

23. A method comprising:

(a) providing a cell which expresses neither CD26 nor CD45 on its surface; and

20 (b) transfecting said cell with a nucleic acid encoding CD26 and a nucleic acid encoding CD45.

24. A method of generating a hybridoma cell, said method comprising:

(a) providing a cell transfected with nucleic acid  
25 encoding CD26, such that said cell expresses CD26 on its surface;

(b) using said cell as an antigen to induce an immune response in a subject animal; and

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(c) fusing a B lymphocyte from said subject animal with a cell from an immortal cell line to produce a hybridoma cell.

25. A hybridoma cell generated by the method of claim 24, wherein said hybridoma cell produces a monoclonal antibody specific for CD26.

26. A cell-free preparation of CD26, or a fragment thereof, complexed with CD45, or a fragment thereof.

27. A polypeptide fragment of CD26 or analog thereof capable of disrupting the naturally-occurring binding interaction between p43 and CD26.

28. A method for screening candidate compounds to identify compounds capable of inhibiting the binding of CD26 to p43, said method comprising the steps of:

(a) providing a first and a second sample of cells expressing both CD26 and p43;

(b) incubating said first sample in the presence of a candidate compound;

(c) incubating said second sample in the absence of said candidate compound;

(d) generating a first immunoprecipitate by adding to said first sample a first aliquot of an anti-CD26 antibody;

(e) generating a second immunoprecipitate by adding to said second sample a second aliquot of said antibody; and

(f) determining whether the amount of p43 present in said first immunoprecipitate is less than the amount of p43 present in said second immunoprecipitate, the presence of a lesser amount of p43 in said first

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immunoprecipitate than in said second immunoprecipitate indicating that said candidate compound inhibits said binding.

29. A purified preparation of p43.

5           30. A method of detecting CD26 mRNA in a cell, said method comprising the steps of:

          (a) providing a biological sample comprising mRNA of a cell;

          (b) contacting said sample with a single-stranded  
10 nucleic acid probe comprising a segment of the sense strand of SEQ ID NO: 1 at least 8 nucleotides in length; and

          (c) detecting hybridization of said probe with said sample, said hybridization indicating the presence  
15 of CD26 mRNA in said cell.

31. A fragment of CD26 in which at least one of the amino acids in the segment Gly627-Gly631 is deleted.

32. The fragment of claim 31, wherein all of said segment is deleted.

20           33. The fragment of claim 32, wherein said fragment has the amino acid sequence shown in SEQ ID NO: 8.

34. A polypeptide fragment of CD26 lacking residues 1-34 of intact CD26.

25           35. The polypeptide fragment of claim 34, wherein said fragment additionally lacks residue 35.

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36. The polypeptide fragment of claim 35, wherein said fragment additionally lacks residue 36.

37. The polypeptide fragment of claim 36, wherein said fragment additionally lacks residue 37.

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1: GACGCCGACGATGAAGACACCGTGGAAGGTTCTTCTGGGACTGCTGGGTGCTGCTGCGCTGTGCAC  
 1: M K T P W K V L L G L L G A A A L V T  
 67: CATCATCACCGTGCCCGTGGTTCTGCTGAACAAGGCACAGATGATGCTACAGCTGACAGTCGCAAAA  
 20: I I T V P V V L L N K G T D D A T A D S R K  
 135: CTTACACTCTAACTGATTACTTAAAAATACTTATAGACTGAAGTTATACTCCTTAAGATGGATTCA  
 42: T Y T L T D Y L K N T Y R L K L Y S L R W I S  
 203: GATCATGAATATCTCTACAAAACAAGAAAAATAATCTTGGTATTCAAATGCTGAATATGGAACAGCTC  
 65: D N E Y L Y K Q E N N I L V F N A E Y G N S S  
 270: AGTTTCTTGGAGAACAGTACATTTGATGAGTTTGGACATTTCTATCAATGATTATTCATATCTCCTG  
 88: V F L E N S T F D E F G H S I N D Y S I S P  
 337: ATGGGCAGTTTATTCTCTTAGAATACAACTACGTGAAGCAATGGAGGCATTCCTACACAGCTTCATAT  
 110: D G Q F I L L E Y N Y V K Q W R H S Y T A S Y  
 404: GACATTTATGATTAAATAAAAGGCAGCTGATTACAGAAGAGAGAGATTCCAAACAACACACAGTGGGT  
 133: D I Y D L N K R Q L I T E E R I P N N T Q W V  
 571: CACATGGTCACCAGTGGGTCATAAAATTGGCATATGTTTGGACAATGACATTTATGTTAAAAATTGAAC  
 156: T W S P V G H K L A Y V W N N D I Y V K I E  
 638: CAAATTTACCAAGTTACAGAATCACAATGGACGGGGAAGAAGATATAATATGGAATAACTGAC  
 178: P N L P S Y R I T W T G K E D I I Y N G I T D  
 705: TGGGTTTATGAAGAGGAAGTCTTCAGTGCCTACTCTGCTGTGGTGTCTCCAAACGGCAGCTTTT  
 201: W V Y E E E V F S A Y S A L W W S P N G T F L

FIG. 1A

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872: AGCATATGCCCAATTAAACGACACAGAAGTCCCACTTATTGAATACTCCTTCTACTCTGTATGAGTCAC  
224: A Y A Q F N D T E V P L I E Y S F Y S D E S  
939: TGCAGTACCCAAAGACTGTACGGGTCCATATCCAAAGGCAGGAGCTGTGAATCCAACCTGTAAGTTC  
246: L Q Y P K T V R V P Y P K A G A V N P T V K F  
1006: TTTGTTGTAATAACAGACTCTCTCAGCTCAGTCAACCAATGCCAACTTCCATACAAATCACTGCTCCTGC  
269: F V V N T D S L S S V T N A T S I Q I T A P A  
1073: TTCTATGTTGATAGGGGATCACTACTTGTGTGATGTGACATGGGCAACACAAAGAAATTTCTTTGC  
292: S M L I G D S Y L C D V T W A T Q E R I S L  
1140: AGTGGCTCAGGAGGATTCAGAACTATTTCGGTCATGGATATTTGTGACTATGATGAATCCAGTGGGAAGA  
314: Q W L R R I Q N Y S V M D I C D Y D E S S G R  
1207: TGAACTGCTTAGTGGCAGCGCAACACATTGAAATGAGTACTACTGGCTGGTGGTGAAGATTAGGCC  
337: W N C L V A R Q H I E M S T T G W V G R F R P  
1274: TTCAGAACCTCATTTTACCCTTGATGGTAATAGCTTCTACAAGATCATCAGCAATGAAGAAGGTTACA  
360: S E P H F T L D G N S F Y K I I S N E E G Y  
1341: GACACATTTGCTATTTCCAAATAGATAAAAAGACTGCACATTTATTACAAAAGGCACCTGGGAAGTC  
382: R H I C Y F Q I D K K D C T F I T K G T W E V  
1408: ATCGGGATAGAAGCTCTAACCCAGTGATTATCTATCTACTACATTAGTAATGAATATAAAGGAATGCCAGG  
405: I G I E A L T S D Y L Y Y I S N E Y K G M P G  
1475: AGGAAGGAATCTTTATAAAAATCCAACTTAGTGACTATACAAAAGTGACATGCCCTCAGTTGTGAGCTGAA  
429: G R N L Y K I Q L S D Y T K V T C L S C E L N  
1542: TCCGGAAAGGTGTCAGTACTATTCTGTGTCTATTCAGTAAAGAGCGGAAGTATTATCAGCTGAGATGTT  
452: P E R C Q Y Y S V S F S K E A K Y Y Q L R C S \*

FIG. 1B

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1609: CGGTCCTGGTCTGCCCTCTATACCTACACAGCAGCGTGAATGATAAAGGGCTGAGAGTCCTGGAAGA  
475: G P G L P L Y T L H S S V N D K G L R V L E D  
1676: CAATTCAGCTTTGGATAAAATGCTGCAGAATGTCCAGATGCCCTCCAAAACCTGGACTTCATATTTT  
498: N S A L D K M L Q N V Q M P S K K L D F I I L  
1743: GAATGAACAACAAATTTTGGTATCAGATGATCTTGGCTCCTCATTTTGATAAAATCCAGAATAATCCTCT  
521: N E T K F W Y Q M I L P P H F D K S K Y P L  
1810: ACTATTAGATGTATGCAGGCCCATGTAGTCAAAAAGCAGACACTGTCTTCAGACTGAACCTGGGCCAC  
544: L L D V Y A G P C S Q K A D T V F R L N W A T  
1877: TTACCTTGCAAGCACAGAAAACATATATAGTAGCTAGCTTTGATGGCAGAGGAAGTGGTTACCAAGGAGA  
567: Y L A S T E N I I V A S F D G R G S G Y Q G D  
1944: TAAGATCATGCAATCAACAGAAAGACTGGGAACATTTGAAGTTGAAGATCAAAATTGAAGCAGCCAG  
590: K I M H A I N R R L G T F E V E D Q I E A A R  
2011: ACAATTTCAAAAATGGGATTGTGGACAACAACGAATTGCAATTTGGGCTGGTCATATGGAGGGTA  
613: Q F S K M G F V D N K R I A I W G W S Y G G Y  
2078: CGTAACCTCAATGGTCCTGGGATCAGGAAGTGGCGTGTTCAGTGTGGAATAGCCGTGGCGCCCTGTATC  
636: V T S M V L G S G S G V F K C G I A V A P V S

FIG. 1C

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2145: CCGGTGGAGTACTATGACTCAGTGTACACAGAACGTTACATGGGTCTCCAACTCCAGAACAACT  
659: R W E Y Y D S V Y T E R Y M G L P T P E D N L  
2212: TGACCATACAGAAATTCAACAGTCAATGAGCAGAGCTGAAAATTTTAAACAAGTTGAGTACCTCCTTAT  
682: D H Y R N S T V M S R A E N F K Q V E Y L L I  
2279: TCATGGAACAGCAGATGATAACGTTCACTTTCAGCAGTCAGCTCAGATCTCCAAAGCCCTGGTCGATGT  
705: H G T A D D N V H F Q Q S A Q I S K A L V D V  
2346: TGGAGTGGATTTCAGGCAATGTGGTATACTGATGAAGACCATGGAAATAGCTAGCAGCACAGCACACCA  
728: G V D F Q A M W Y T D E D H G I A S S T A H Q  
2413: ACATATATATACCCACATGAGCCACTTCATAAAACAATGTCTCTTTACCTTAGCACCTCAAAATACC  
751: H I Y T H M S H F I K Q C F S L P  
2480: ATGCCATTTAAAGCTTATTAAACTCATTTTGTCTTTCATTATCTCAAAACTGCACGTCAAGATGATG  
2547: ATGATCTTTAAATAACACACTCAATCAAGAACTTAAGTTACCTTGTTCCTCCAAATTTCAATACCTAT  
2614: CATCTTAAGTAGGACTTCTGTCTTCAACACAGATTATACCTTACAGAAGTTTGAATTTATCCGGTCGG  
2681: GTTTTATGTTTAAATCATTTCTGCATCAGCTGCTGAAAACAACAAATAGGAATGTTTATGGAGGC  
2748: TTTGCATAGATTCCCTGAGCAGGATTTTAATCTTTTCTAAGTGGACTGGTCAAAATGTTCTCTTC  
2815: TTTAAAGGATGGCAAGATGTGGCAGTGTCACTAGGCGGACAGGATAAGAGGGATAGGGAG  
2882: AGAGATAGCAGGCGATGGCTGGGAACCCAAAGTCCAGCATACCAACAGCAGGCTACTGTCAGCTC  
2919: CCCTCGGAGAAAACGTGTCAGTCTGCGTGTGAACAGCTCTTCTCTTTAGAGCACAATGGATCTCGAGG  
3016: GATCTTCCATACCTACCAGTTCTGCGCCTCGAGGCGCGACTCTAGA 3063

FIG. 1D

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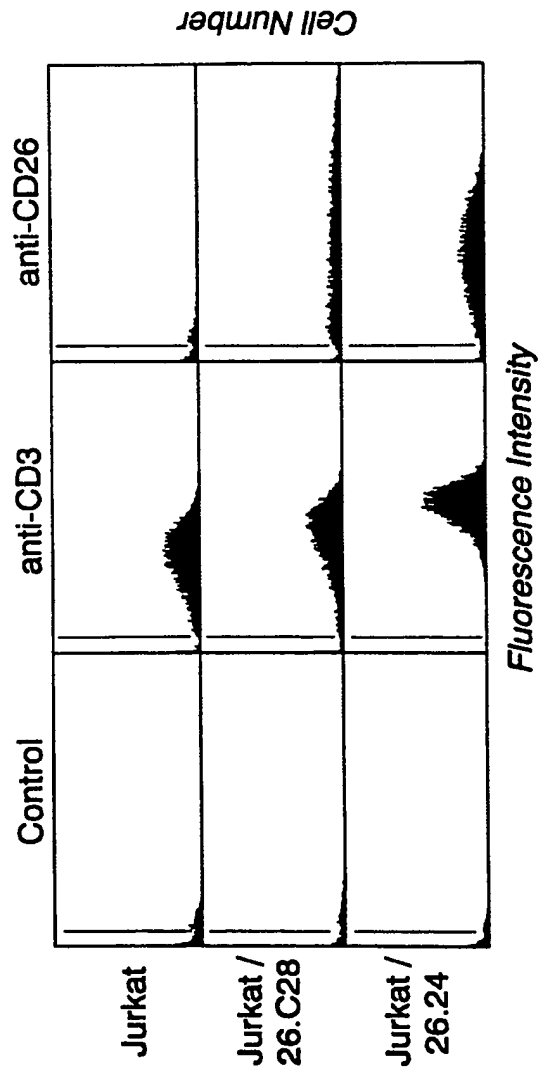


FIG. 2

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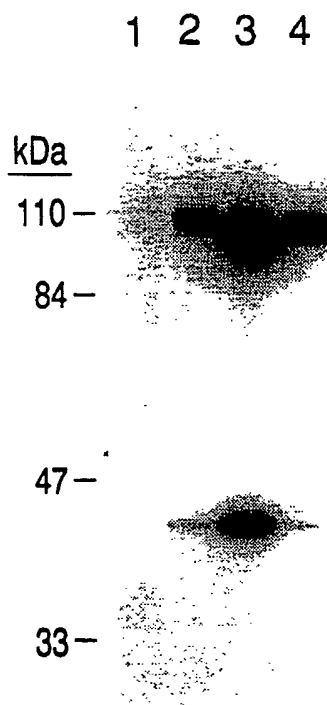


FIG. 3A

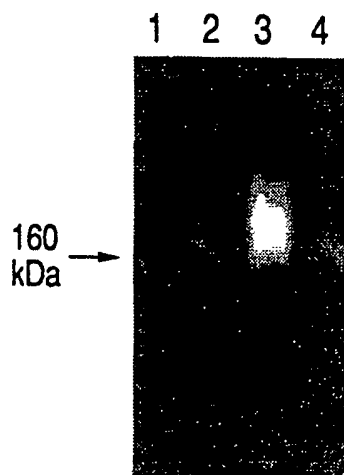


FIG. 3B



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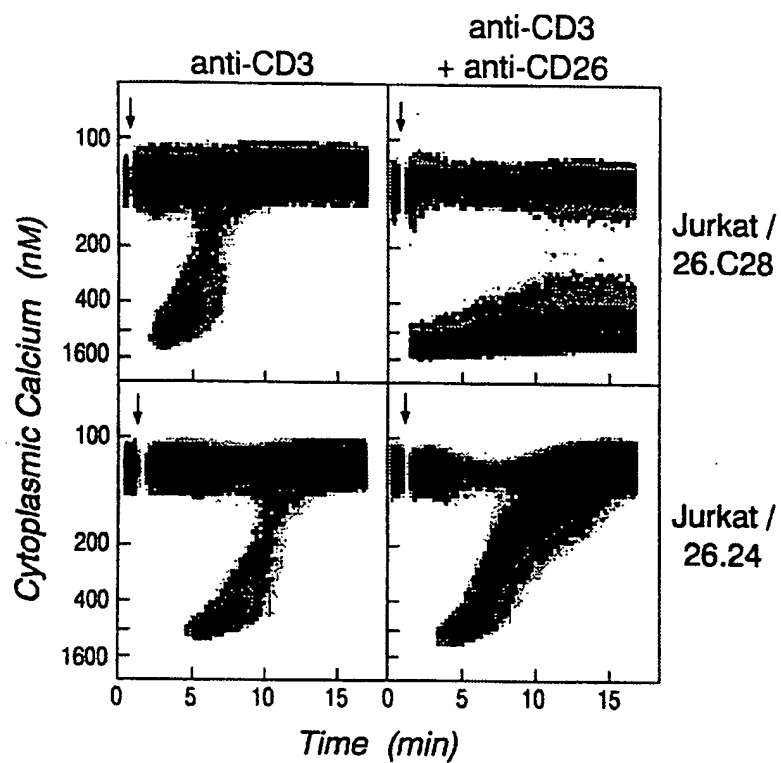


FIG. 4

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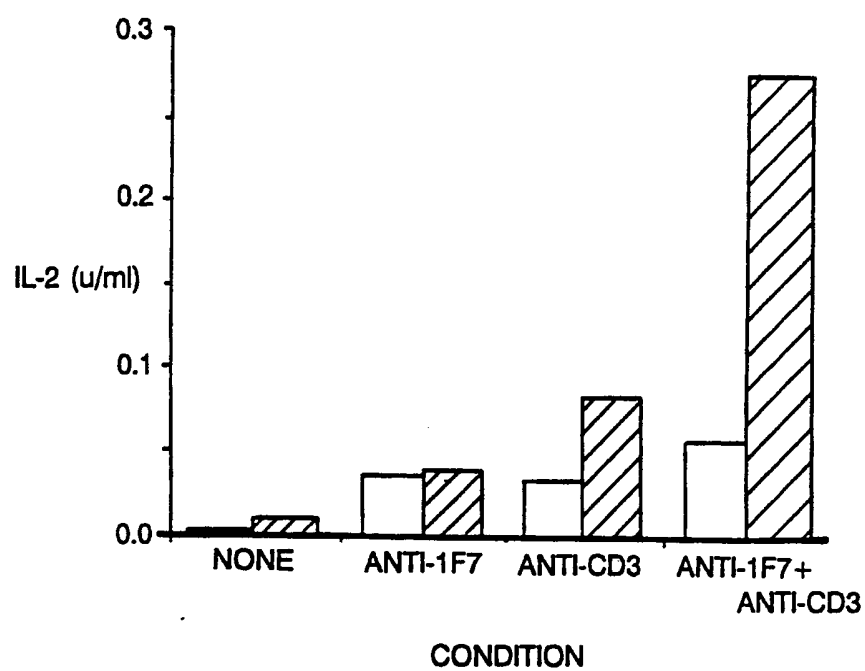


FIG. 5

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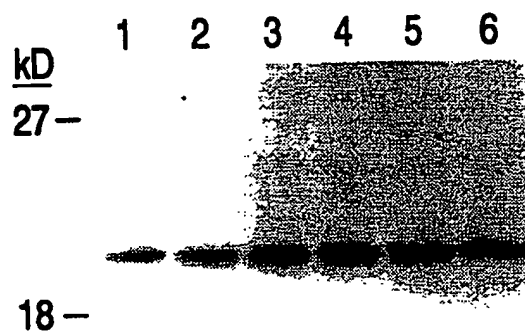


FIG. 6

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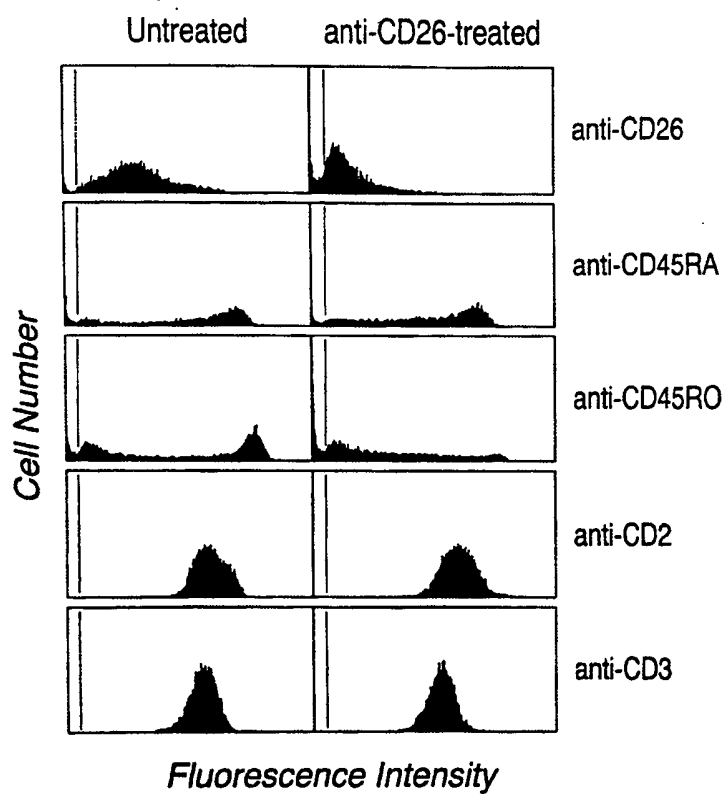


FIG. 7

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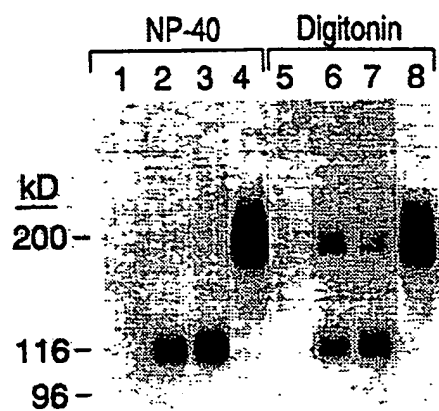


FIG. 8

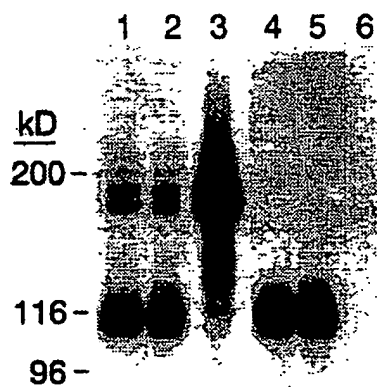


FIG. 9

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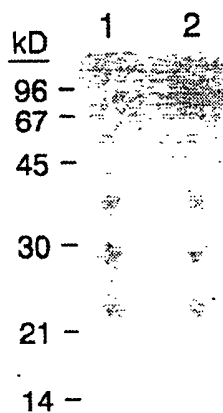


FIG. 10

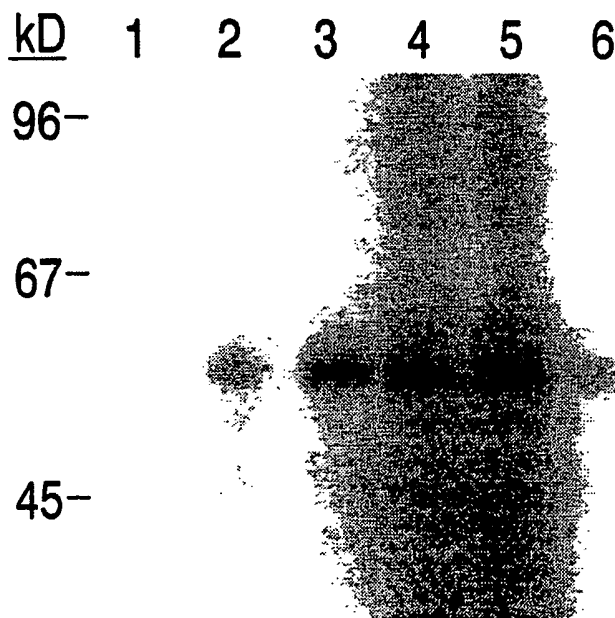


FIG 11  
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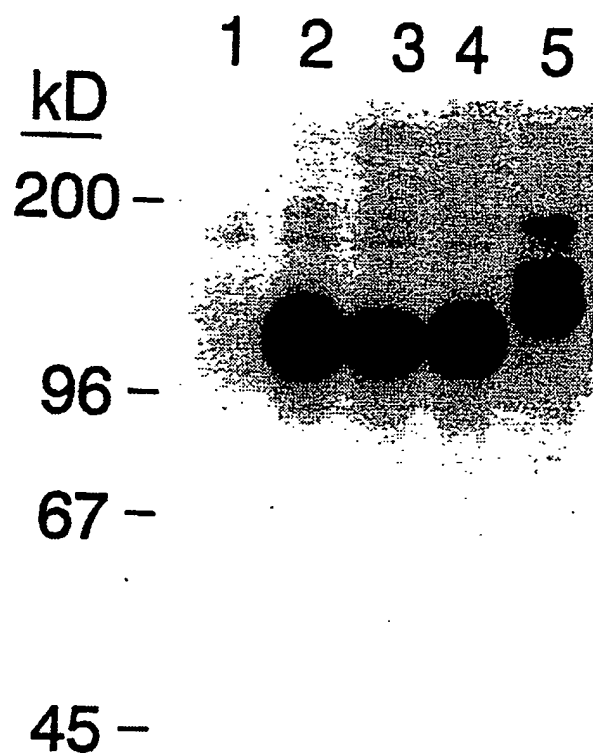


FIG. 12

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1 CD26: MKTPWKVLLGLLGAALVTIITVPVLLNKGTTDADSRKTYTLTDYLNKNTYRLKLYSL 51  
 ↓↓↓  
 101  
 RWISDHEYLYKQENNILVFNAEYGNSSVFLENSTFDEFGHSINDYSISPDGQFILLEYN  
 151  
 VKQWRHSYASDYIDLNKRQLITEERIPNNTQWVTWSPVGHKLAYVWNNDIYVKIEPNL  
 201  
 PSYRITWTGKEDIINYGITDWVYEEVEFSAYSALWSPNGTFLAYAQFNDTEVPLIEYSF  
 251  
 YSDESQYPKTVRPYPKAGAVNPTVKFFVVNTDSLSSVTNATSIQITAPASMLIGDHYL  
 301  
 CDVTWATQERISLQWLRRIQNYSVMDICDYDESSGRWNCLVARQHIEMSTTGWVGRFRPS  
 401  
 EPHFTLDGNSFYKIIISNEEGYRHICYFQIDKKDCTFITKGTWEVIGIEALTSDYLYYISN  
 451  
 EYKGMPPGGRNLYKIQLSDYTKVTCLSCELNPERCQYYSVSFSKEAKYYQLRCSGPGPLPY  
 501  
 TLHSSVNDKGLRVLEDNSALDKMLQNVQMPSKLDFIILNETKFWYQMLPPHFDKSKKY  
 551  
 PLLLDVYAGPCSQKADTVFRLNWA TYLASTENIIVASFDRGSGYQGDKIMHAINRRLGT  
 601  
 FEVEDQIEAARQFSKMGFVDNKRIAINGWSYGGYVTSMVLGSGSGVFKCGIAVAPVSRWE  
 701  
 YYDSVYTERYMG LPTPEDNLDHYRNSTVMSRAENFKQVEYLLIHGTADDNVHFQQSAQIS  
 751  
 KALVDVGVDFOAMWYTDEDHG IASSTA HQHIYTHMSHF I KQCFSLP

**FIG. 13**



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1  
 D26: MKTPWKVLLGLLGAALVTIITV PVVLLNKGTD ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ATADSRKTYTLTDYLKNTYRLKLYSL  
 RWISDHEYLYKQENNILVFNAEYGNSSVFLENSTFDEFHGSINDYSISPDGQFILLEYN  
 VKQWRHSYASYDIYDLNKRQLITEERIPNNTQWVTWSPVGHKLAYVWNNDIYVKIEPNL  
 PSYRITWTGKEDIYNGITDWVYEEVEVFSAYSALWSPNGTFLAYAQNDETEVPLIEYSF  
 YSDESLQYPKTVRPYPKAGAVNPTVKFFVNTDSLSSVTNATSIQITAPASMLIGDHYL  
 CDVTWATQERISLQWLRRIQNSVMDICDYDESSGRWNCLVARQHIEMSTTGWVGRFRPS  
 EPHFTLDGNSFYKIIISNEEGYRHICYFQIDKKDCTFITKGTWEVIGIEALTS DYLYYISN  
 EYKGMPPGGRNLYKIQLSDYTKVTCLSCELNPERCQYYSVSFSKEAKYYQLRCSGPGGLPLY  
 TLHSSVNDKGLRVLEDNSALDKMLQNVQMPKSKLDFFIILNETKFWYQMLPPHFDKSKKY  
 PLLLDVYAGPCSQKADTVFRLNWTATYLASTENIIVASFDRGSGYQGDKIMHAINRRLGT  
 FEVEDQIEAARQFSKMGFVDNKRIAIWGSYGGYVTSMVLGSGGVFKCGIAVAPVSRWE  
 YYDSVYTERYMG LPTPEDNLDHYRNSTVMSRAENFKQVEYLLIHGTADDNVHFQQSAQIS  
 KALVDVGVDVFQAMWYTDDEHGIASSTAQHIIYTHMSHFIKQCFSLP

FIG. 14

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1  
CD26: MKTPWKVLLGLLGAAALVTIITVPVLLNKGTDATADSRKTYTLTDYLNKNTYRLKLYSL 51

101  
RWISDHEYLYKQENNILVFNAEYGNSSVFLENSTFDEFGHSINDYSISPDGQFILLEYN 101

151  
VKQWRHSYTASYDIYDLNKRQLITEERIPNNTQVWTWSPVGHKLAYVWNNDIYVKIEPNL 151

201  
PSYRITWTGKEDIYNGITDWVYEEEEVSAYSALWSPNGTFLAYAQFNDTEVPLIEYSF 201

251  
YSDESLQYPKTVRVPYPKAGAVNPTVKFFVNTDSLSSVTNATSIQITAPASMLIGDHYL 251

301  
CDVTWATQERISLQWLRRIQNYSVMDICDYDESSGRWNCLVARQHIEMSTGTGWVGRFPS 301

401  
EPHFTLDGNSFYKIIISNEEGYRHICYFQIDKKDCTFITKGTWEVIGIEALTSDYLYISN 401

451  
EYKGMPPGGRNLYKIQLSDYTKVTCLSCELNPERCQYYSVSFSKEAKYYQLRCSGPGLPLY 451

501  
TLHSSVNDKGLRVLEDNSALDKMLQNVQMPKSKLDFIILNETKFWYQMLPPHFDKSKKY 501

551  
PLLLDVYAGPCSQKADTVFRLNWTATYLASTENIIVASFDRGRSGYQGDKIMHA 551

FIG. 15

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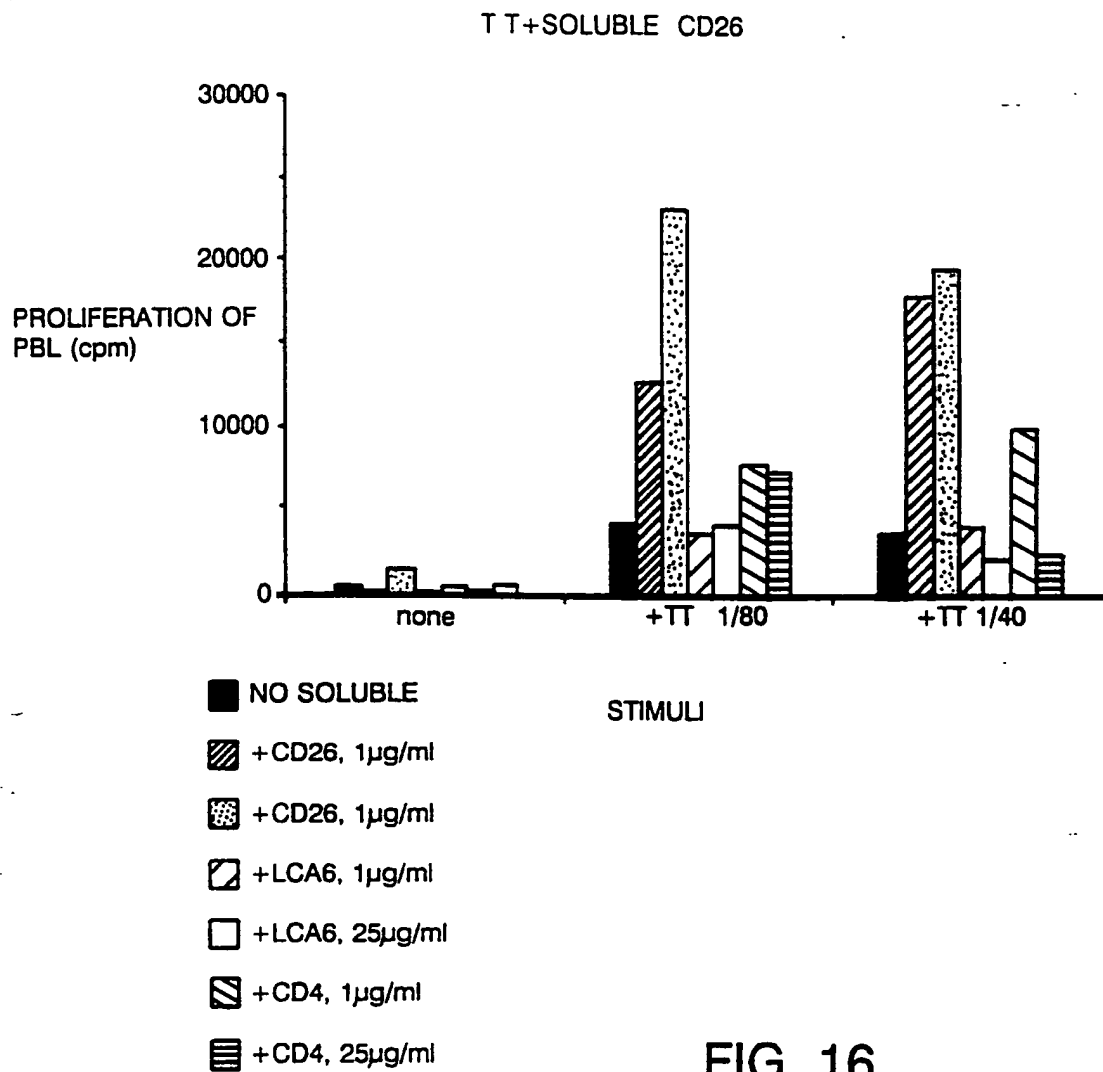


FIG. 16

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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US92/02892

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : C07K 3/28, 7/06, 15/28; C12N 15/00, 15/02, 15/11; G01N 33/50

US CL :

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. :

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EMBL Data Library, issued 16 July 1991, Y. Misumi et al., "Primary structure of human liver dipeptidyl peptidase IV", accession number HSPCHDP7, entire document.	1, 3, 6, 10, 17, 19, 20-25, 30-37
Y	Journal of Biological Chemistry, Volume 263, Number 32, issued 15 November 1988, W. Hong et al, "Membrane orientation of rat gp110 as studied <u>in vitro</u> translation, pages 16892-16898, see Fig. 1.	2, 4, 5, 9, 11-13
Y	Journal of Immunology, Volume 147, Number 8, issued 15 October 1991, Y. Torimoto et al, "Coassociation of CD26 (dipeptidyl peptidase IV) with CD45 on the surface of human T lymphocytes", Abstract	14-17, 26, 28
Y	E. Harlow et al, "Antibodies: A Laboratory Manual" published 1988 by Cold Spring Harbor Laboratory (N.Y.), see pages 148-151, 158-159, 207-219, entire document.	16, 24, 25
Y	Genomics, Volume 10, issued 1991, J. L. Fernandez-Luna et al, "Characterization and expression of the human leukocyte-common antigen (CD45) gene contained in yeast artificial chromosomes", pages 756-763, especially pages 760-761.	18-23

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be part of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A*	document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means		
*P* document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

08 OCTOBER 1992

Date of mailing of the international search report

27 OCT 1992

Name and mailing address of the ISA/  
Commissioner of Patents and Trademarks  
Box PCT  
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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US92/02892

### B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

CAS, APS, MEDLINE, BIOSIS

search terms: CD26, CD45, clone, immunoprecipitation, coprecipitation, interaction, cell surface, dipeptidylpeptidase IV, antigens, activation antigen, peptide fragments, p43, polypeptide, plasmid

### BOX I. OBSERVATIONS WHERE CLAIMS WERE FOUND UNSEARCHABLE

2. Where no meaningful search could be carried out, specifically:

These claims read on the polypeptide fragments of CD26 represented by SEQ ID Numbers: 2 and 3, said polypeptide being soluble under physiological conditions. Both of these polypeptides are hydrophobic and would not be soluble under physiological conditions. It appears that applicants intended to claim the polypeptide fragments of CD26 minus the polypeptide fragments represented by SEQ ID Numbers: 2 and 3.

# INTERNATIONAL SEARCH REPORT

International application No.

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## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 27  
because they relate to subject matter not required to be searched by this Authority, namely:  
  
The claim reads on naturally occurring CD26.
2. ☒ Claims Nos.: 7 and 8  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
  
Please See Extra Sheet.
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐  
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US92/02892

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Scandinavian Journal of Immunology, Volume 34, Number 2, issued August 1991, M. T. Ferm et al, "Human MHC class I antigens are associated with a 90-kDa cell surface protein.", Abstract.	28-29
Y	Immunogenetics, Volume 25, issued 1987, C. LeGuern et al, "Sequence determination of a transcribed rabbit class II gene with homology to HLA-DQ <sub>α</sub> ", page 104-109, see pages 106 and 107 and attached sequence alignment.	28-29

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